NUTRITION & HEALTH

Preventive Nutrition

The Comprehensive Guide for Health Professionals

THIRD EDITION

Edited by

Adrianne Bendich, PhD, FACN

Richard J. Deckelbaum, MD, FRCP(C)

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Third Edition

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Adrianne Bendich, Series Editor

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Dedication

AB dedicates this volume to her husband, David Kafkewitz, PhD,
wth deepest gratitude for his unconditional support and understanding.

Richard J. Deckelbaum dedicates this volume to William and Katya,
and the rest of our family and friends.

Series Editor's Introduction

The *Nutrition and Health Series* of books has had great success because each volume has the consistent overriding mission of providing health professionals with texts that are essential since each includes (1) a synthesis of the state of the science; (2) timely, in-depth reviews by the leading researchers in their respective fields; (3) extensive, up-to-date, fully annotated reference lists; (4) a detailed index; (5) relevant tables and figures; (6) identification of paradigm shifts and their consequences; (7) virtually no overlap of information between chapters, but targeted, interchapter referrals; (8) suggestions of areas for future research; and (9) balanced, data-driven answers to patient as well as health professionals' questions that are based on the totality of evidence rather than the findings of any single study.

The series volumes are not the outcome of a symposium. Rather, each editor has the potential to examine a chosen area with a broad perspective, both in subject matter as well as in the choice of chapter authors. The editors, whose trainings are both research- and practice-oriented, have the opportunity to develop a primary objective for each book, define its scope and focus, and then invite the leading authorities to be part of their initiative. The authors are encouraged to provide an overview of the field, discuss their own research and relate the research findings to potential human health consequences. Because each book is developed *de novo*, the chapters are coordinated so that the resulting volume imparts greater knowledge than the sum of the information contained in the individual chapters. Many of the books in the series come with a complementary copy of the book on a CD; recently published books are available as e-books and individual chapters of the e-books are also now available from Humana Press' website, Humanapress.com

Preventive Nutrition: The Comprehensive Guide to Health Professionals, Third Edition, edited by me and Richard Deckelbaum remains the flagship volume of the series and continues to improve with each edition. The first edition, published in 1997, was widely acclaimed for its emphasis on data-driven recommendations based on the totality of the evidence that included the newest scientific discoveries and clinical applications in the key areas of preventive nutrition: cancer, cardiovascular disease, osteoporosis, age-related eye diseases, immune function, birth outcomes, and an international perspective on preventive nutrition strategies. The second edition, published in 2001, provided critical updates and also included new information on nutrition-related disease prevention strategies. In the same year, Richard and I also published a volume, *Primary and Secondary Preventive Nutrition,* that reviewed several other clinical areas including obesity and diabetes. In response to our readers and reviewers, we have combined the most relevant and timely chapters from these two volumes into the third edition of *Preventive Nutrition.*

Preventive Nutrition, Third Edition, which is now more than 900 pages of text, contains 37 chapters that help to define the areas where health professionals can improve health and reduce the risk of disease with dietary modifications. We have enhanced the

contents of the first two editions even further with the addition of 16 new chapters as well as comprehensive updates of all of the chapters that are included in this edition. Chapter authors are the most authoritative leaders in their various fields of expertise. The volume contains cutting-edge chapters from 79 authors who provide a truly global perspective to preventive nutrition opportunities. In addition, Dr. Alfred Sommer, whose international research has been honored with the prestigious Lasker Award, has provided a thoughtful Foreword for our book. Thus, Richard and I have worked diligently to develop a third edition that is destined to be the benchmark in the field because of its extensive, in-depth chapters covering the most important aspects of preventive nutrition with emphasis on normal human physiology and nutritional needs, analyses of epidemiological findings, in-depth and balanced discussions of clinical findings including the role of nutrients in disease treatment as well as primary and secondary disease prevention.

The book chapters are logically organized in nine sections to provide the reader with a basic understanding of the role of preventive nutrition strategies: I. Preventive Nutrition Overview, II. Cancer Prevention, III. Cardiovascular Disease Prevention, IV. Diabetes and Obesity, V. Bone Diseases, VI. Prevention of Major Disabilities: Improvement in Health Outcomes, VII. Optimal Pregnancy/Infancy Outcomes, VIII. Preventive Nutrition: Global Perspectives, and finally, IX. Critical Issues for the 21st Century. This logical sequence of chapters provides the latest information on the current standards of practice for clinicians, related health professionals including the dietitian, nurse, pharmacist, physical therapist, behaviorist, psychologist, and others involved in the team effort required for successful treatments. This comprehensive volume also has great value for academicians involved in the education of graduate students and postdoctoral fellows, medical students and allied health professionals who plan to interact with patients on questions concerning the science behind the latest "study" presented on the evening news because the chapters present a synthesis of the totality of the evidence, not a conclusion based on a single study.

In-depth, balanced reviews of the current science behind questions related to topics such as soy; vitamin supplements; fat substitutes; DHA; trans-fatty acids; carotenoids, such as lycopene and lutein; and numerous other dietary components, provide health care professionals and students with excellent, well-referenced answers. Medically related professionals will certainly find the chapters dealing with obesity across the age span, and including the genetic components of related diseases, of particular importance to their practice. Additional chapters provide in-depth reviews of the functions of vitamins and minerals and other dietary factors in the endocrine, cardiovascular, respiratory, gastrointestinal, immune, skeletal, and ocular systems of the body. Obesity is a critical concern for virtually every health professional. The third edition has an expanded section on obesity and diabetes that includes important aspects of childhood obesity. There is a clear, data-driven message throughout the obesity section of the volume that obesity is a chronic disease. The volume is of particular importance because it includes understandable chapters in highly technical areas written by the leading authorities who are actually working in the areas of research discussed in their chapters.

Of added value to health care professionals who are interested in public health is the exceptional introductory chapter that places preventive nutrition in its historic perspective and goes on to project the enormous potential for preventive strategies to reduce

human suffering. This chapter is balanced with the final book chapter that provides a practical approach to determining the economic value of adopting a number of the preventive nutrition strategies discussed throughout the volume. An often-overlooked dietary component, alcohol, is critically examined in a separate chapter, as it can well be a double-edged sword and can impact diseases and conditions that would otherwise be beneficially affected by implementing preventive nutrition strategies. Other unique chapters examine the national differences in the consumer messages that are permitted for dietary factors and the current status of nutrition education in medical schools. Of great importance, the authors have provided chapters that balance the most technical information with discussions of its importance for clients and patients as well as graduate and medical students, health professionals, and academicians.

The third edition of *Preventive Nutrition* continues to serve as a major resource on international nutrition because the volume includes important chapters about implementation strategies from leading experts about national interventions in Europe, Asia, and Africa. Prior editions were so well accepted by those interested in the impact of nutrition on world health, in part because of this unique section on global perspectives. In the current edition, this section examines the successes and consequent public health implications of national preventive nutrition strategies, not only in the United States and Europe, but also in "Westernizing" nations and developing countries. Updates from Norway and Chile that were first provided in the 1997 edition are now included in the third edition. Also updated is the chapter that examines the potential health benefits to the United States with adoption of preventive nutrition strategies. There are two insightful chapters on the growing presence of obesity in Latin America and Japan and how the Westernization of eating habits has negatively impacted the health of these nations.

Hallmarks of all chapters include bulleted key points at the beginning of each chapter as well as a detailed table of contents; complete definitions of terms with the abbreviations fully defined for the reader, and consistent use of terms between chapters. There are numerous relevant tables, graphs, and figures as well as up-to-date references; all chapters include a conclusion section that provides the highlights of major findings. Each chapter ends with a recommendations section that helps to guide the reader to data-driven, balanced advice for their students, clients, and/or patients. The volume contains a highly annotated index and within chapters, readers are referred to relevant information in other chapters.

In conclusion, *Preventive Nutrition: The Comprehensive Guide for Health Professionals, Third Edition* provides health professionals in many areas of research and practice with the most up-to-date, well-referenced and easy-to-comprehend volume on the importance of understanding the current strategies for enhancing health and preventing disease through the implementation of well-accepted and highly recommended preventive nutrition options. This volume will serve the reader as the benchmark in the nutrition field, as it stands as the most authoritative resource to date on prevention, and is a very welcome addition to the *Nutrition and Health Series*.

Adrianne Bendich, *PhD, FACN*
Series Editor

Foreword

Why a third edition of *Preventive Nutrition*, and only a few years after the second? Because the public has developed an insatiable appetite for advice on what foods to eat and supplements to take, a phenomenon fed in large part by the sensationalist hype every new "study" receives from the media. And the media are being fed with tantalizing new data and claims at an accelerating rate.

Unfortunately, although people and policymakers want definitive recommendations, the vast outpouring of new data arrives in fragmentary (and often contradictory) bites, far too messy for the individual health professional to assimilate. By synthesizing these latest data and integrating them into the broad body of existing information, *Preventive Nutrition* goes a long way toward orienting health professionals to the field and guiding the advice they give their patients and the public.

No one can seriously doubt the impact that nutrition has on health—well beyond the classical deficiency syndromes described in the past (xerophthalmia, anemia, scurvy, pellagra). Recognition of the role of saturated fats in coronary artery disease led to a dramatic change in America's dietary habits and a halving of the incidence of fatal heart attacks. Public health interventions don't get much better than that.

More recently, the surprising discovery that preconceptional folate supplements could significantly reduce the incidence of neural tube defects resulted in the mandatory fortification of cereal flour, which led to a significant reduction in the incidence of this devastating congenital defect. An outpouring of new studies suggest potential benefits from a host of nutritional interventions for chronic diseases—critically important, given greater longevity and aging of virtually all the world's populations. Some, like the need for increased calcium to reduce the soaring rates of osteoporosis, are well established, even if the ideal formulation (and concurrent use of drugs to the same end) is still being studied and the recommendations refined. Others, like zinc and antioxidant supplementation to retard progression of macular degeneration, the leading cause of irreversible blindness, now have the support of a rigorous randomized trial, which also, importantly, defined the small, specific group in which this proved beneficial.

But not all the "news" is necessarily true—at least not the way it is first perceived. Many of our most important and much-ballyhooed insights arise from observational studies: people who eat more salads and vegetables purportedly have lower risks of certain cancers; those consuming more fiber appear to have a lower risk of colon cancer. Although that might be so, randomized clinical trials have failed to demonstrate that changing the intake of these dietary factors actually altered the risk of disease. The "best available evidence" is often less than we would prefer; and, as is often the case, recommendations and policies are made with less than adequate data.

Of course some "truths" don't require randomized trials, but what we do about them do. Today's growing epidemic of obesity, already fueling a secondary epidemic of "adult"

onset of diabetes among adolescents, is clearly fueled by increased intake of calorie-dense foods coupled with a sedentary lifestyle—and not just in the developed world. But instead of reducing the amount of food they consume and getting more exercise, inaccurately referred to as "simple lifestyle changes," the small part of the public that actually cares at all about its nutritional health and waistline has fixated instead on elaborate new dietary fetishes, whether "Atkins," "South Beach," or "The Zone." Do these really work? Are they safe? How should health professionals and policymakers recommend intercepting our collective corpulence in ways that are proven safe and effective for the populace as a whole?

The plethora of information emanating from molecular biology on the one hand and epidemiological and clinical studies on the other have provided, and will ultimately provide, the basis for rational, evidence-based nutritional "prescriptions" for better health. People seek (and deserve) the best advice available from their health professionals. Given the growing numbers of chronic diseases and the direct and indirect costs associated with them, relatively inexpensive nutritional interventions need to be discovered, promulgated, and actually employed. This latest edition of *Preventive Nutrition* provides a comprehensive integration of the best available data on the role of nutrition in health. It is an excellent place to discover what still needs scientific inquiry and what health professionals might reasonably advise their patients and the public.

Alfred Sommer, *MD, MHS*
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Preface

Preventive Nutrition was first published in 1997; the second edition appeared in 2001. Also in 2001, we edited a volume entitled *Primary and Secondary Preventive Nutrition*. Both texts were very well accepted as critical resources for medical school libraries and health care professionals including physicians, nutritionists, dietitians, nurses, and pharmacists. The volumes were also used by many academic researchers as the basis for developing graduate courses in preventive nutrition; *Preventive Nutrition* has been adopted as a text in many of the most highly rated graduate nutrition programs in the United States, Europe, South America, and Asia. The overall goal of the third edition of *Preventive Nutrition* is to combine and update chapters of excellence that were included in the first and second editions of *Preventive Nutrition*, as well as critical chapters from *Primary and Secondary Preventive Nutrition* and to add new chapters that emphasize the critical role of nutrition in a growing number of health arenas.

In the third edition we provide health professionals with up-to-date, comprehensive reviews that evaluate the dietary practices and interventions that have been shown to reduce disease risk and/or to improve health outcomes. Evidence-based nutritional interventions are a critical component of preventive medicine approaches to prevent an increasing number of diseases and diminish their consequences. Within the past decade, many studies have demonstrated the potential for optimizing macro- and micronutrient intakes to improve not only individual outcomes throughout the life cycle, from fetal development to the aging process, but also to reduce individual and national health care costs.

This third edition is more comprehensive that the previous volumes. The average number of chapters for each of the three previous volumes was 25; this edition contains 37 chapters. Several chapters that first appeared in *Primary and Secondary Preventive Nutrition* have been revised and are therefore new subject areas for *Preventive Nutrition, Third Edition*. The volume is divided into the following nine sections: I. Preventive Nutrition Overview; II. Cancer Prevention; III. Cardiovascular Disease Prevention; IV. Diabetes and Obesity; V. Bone Diseases; VI. Prevention of Major Disabilities: Improvement in Health Outcomes; VII. Optimal Pregnancy/Infancy outcomes; VIII. Preventive Nutrition: Global Perspectives, and finally, IX. Critical Issues for the 21st Century. The numbers of chapters in each section are well balanced, contain numerous comprehensive tables and clearly labeled figures, and reflect the major areas of recent research important to health professionals. Also new to this edition is the inclusion of Key Points at the beginning of each chapter that define the chapter's critical learning points for the reader. The third edition of *Preventive Nutrition* continues to include a comprehensive index as well as helpful listings of relevant books, journals, and websites.

There is a new introductory chapter that provides an historic perspective as well as insight into the future economic consequences of adopting a preventive nutrition strategy to improve health. In the section on cancer, there is a comprehensive updating of the

components in the diet that affect cancer risk, including an overview on information relating to cancers of the esophagus and stomach. There are new chapters on tomatoes, lycopene and prostate cancer, soy and cancer prevention, and dietary supplement use and their effects on cancer risk. The "new" research on dietary components that are not considered to have traditional "essential nutritional value" but have been shown to have important health consequences, such as fiber, specific long-chain fatty acids, non-provitamin A carotenoids, and other phytochemicals, are placed in perspective with regard to the available knowledge to date by expert authors who are the leaders in their fields.

In the cardiovascular disease section, there are comprehensive revisions to the chapters on iron, homocysteine, and the long-chain omega-3 fatty acids. Within the past decade, there have been several critical intervention studies that examined the potential for antioxidants from supplements to reduce cardiovascular disease risks as well as myocardial infarction, vascular pathology, and ultimately mortality. A new chapter reviews the totality of the evidence on the effects of antioxidant supplements on cardiovascular outcomes.

Diabetes and obesity are major health concerns that are significantly impacted by diet and dietary components. We have included this key section from the *Primary and Secondary Preventive Nutrition* volume as well as five distinctive chapters by leading clinical authorities in obesity and diabetes research. There is a comprehensive chapter on trans fats; another that addresses the current epidemic of childhood and adolescent obesity and insulin resistance followed by two provocative chapters on prevention of childhood obesity—one that examines clinical programs, the other with a more public health perspective. Lastly, there is a chapter on the impact of weight reduction on decreasing chronic disease-related risk factors.

Bone diseases affect millions of adults and not only seniors. We include separate chapters on osteoarthritis (a new chapter) and osteoporosis, and another new chapter on anti-osteoporosis medications . The latter stresses the requirements for calcium, vitamin D, and other nutrients to achieve the full efficacy of these drugs.

In the section on the prevention of major disabilities, there is a new chapter on cataract prevention and the role of antioxidants. There are updated chapters on the importance of antioxidant nutrients in the prevention of diseases resulting from oxidative stress, another that includes recent studies on the importance of micronutrients in maintaining the immune function of healthy older adults, and an updated chapter on the impact of vitamin A on the immune function of the very young, stressing the developing countries. A new chapter reports on recent studies that explored the potential for a century-old intervention, cod liver oil, to reduce the risk of respiratory infections in urban children from the United States.

Another major objective of this volume is to examine key research linking nutritional status with the prevention of birth defects and optimization of birth outcomes. This area is gaining in importance as birth weight and size are being linked to risk of chronic diseases in adulthood. One of the critical nutrient-based discoveries of the last century is the ability to prevent neural tube birth defects by folic acid supplementation. The updated chapter contains the latest data that confirm and strengthen the original findings that periconceptional supplementation with a folic acid-containing prenatal supplement significantly reduced the risk of first time neural tube birth defects as well as cardiovascular

and other serious birth defects. There is a revised chapter on the significant association between micronutrient status and risk of preterm delivery and low birth-weight outcomes. This chapter is complemented by the new chapter on the relevance of measures of maternal thinness for predicting economic crisis in a nation. The final chapter in this section deals with an update of another of the major nutritional advances of the last few decades, the essentiality of long-chain omega-3 fatty acids for the optimal development of the brain and the retina of the fetus and the vision and intellectual capacity of the newborn.

Preventive Nutrition has been so well accepted by those interested in the impact of nutrition on world health in part because of the unique section on global perspectives. This section examined the successes and consequent public health implications of national preventive nutrition strategies, not only in the United States and Europe, but also in "Westernizing" nations and developing countries. A number of these chapters did not appear in the second edition. We have gone back to the authors of these valuable chapters. We are very pleased to include updates of data from Norway and Chile that were first provided in the 1997 edition, and are now included in the third edition. Also updated is the chapter that examines the potential health benefits to the United States with adoption of preventive nutrition strategies. There are two insightful chapters on the growing presence of obesity in Latin America and Japan and how westernization of eating habits has negatively impacted the health of these nations.

The final section of this comprehensive volume is also new to *Preventive Nutrition* and examines critical nutritionally related issues for the 21st century. There is an in-depth look at the role of alcohol consumption and whether the benefits outweigh the risks. Another important addition to the volume is the comprehensive chapter that examines the effects of drug–nutrient interactions from many different perspectives. These interactions become increasingly important as our society ages and uses many drugs daily, all of which have some impact on nutritional status. The last three chapters serve as very useful resources to many of our readers. In the United States, the government regulation of foods and dietary supplements is complex and evolving; thus we include a chapter on the current status of the regulations and the health claims that have emerged in the last decade. Many of our readers are involved in the development of curricula for graduate and medical schools, and there is a chapter on the current level of nutrition education provided in the US medical schools. Finally, we include a chapter that examines the link throughout the life cycle between health and economic value of preventive nutrition strategies in individuals, communities, and/or nations. This last chapter completes the circle and brings us back to the very first chapter that described the historic perspective on nutrition strategies that prevent chronic diseases and improve human health.

The third edition provides access to practicing health professionals, including physicians, nutritionists, dentists, pharmacists, dietitians, health educators, policymakers, and research investigators to the considerable recent research demonstrating that the onset of major diseases can be prevented, or at least delayed, with simple nutritional approaches. Many health professionals are frequently asked about recent findings on vitamins or other nutrients presented in their local newspaper or on the evening news. Patients demand counseling from their health care providers on β-carotene, lycopene, antioxidants, soy, fiber, folate, calcium, and the myriad of bioactive phytochemicals, such as those found in garlic and other foods.

As the demographics of US and European populations change and become more multicultural, it is increasingly important for health professionals to understand the nutritional backgrounds and diversities of their patients and clients. As important, there may be significant national dietary initiatives that provide roadmaps for effective implementation of preventive nutrition within an overall strategy of health improvement, especially for vulnerable members of the population, such as the poor. As editors, we have endeavored to include the most compelling areas of nutritional research that can have immediate impact on the health and education of our readers. We anticipate that the third edition of *Preventive Nutrition* will be a valued resource to health professionals and students who are interested in reducing disease risk and enhancing health by implementing the well-documented preventive nutrition strategies outlined in this volume.

We thank Paul Dolgert and the staff at Humana Press for their confidence in our vision and their assistance in bringing this volume to fruition. We also thank Nicole Ticknor at GSK and Randy Weiss at Columbia University for their administrative support. We remain grateful to our authors for their insightful chapters and to Dr. Alfred Sommer for his eloquent Foreword.

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I Preventive Nutrition Overview

1 Preventive Nutrition

A Historic Perspective and Future Economic Outlook

Nancy D. Ernst and J. Michael McGinnis

KEY POINTS

- Diet and physical inactivity rank first or second in actual causes of death and the only causes that are a daily factor for the entire US population.
- The increasing US life expectancy, which was at an all-time high of 77.2 yr in 2001, signals that prevention of heart disease, cardiac arrest, stroke, and diabetes is a societal concern and that preventive nutrition action can help assure that aging does not predict a worsening state of economic well-being and health.
- Mathematical models (which consider federal nutrition projects such as food labeling, food standards, and dietary guidelines) show economic benefit from dietary changes for a healthier diet. New nutrition label reference values and recommended nutrient fortification guidelines, developed by the Institute of Medicine and soon to be considered by regulatory agencies in the United States and Canada, are also likely to affect dietary change.
- The food market has responded—with innovation, technology, and advertising and has enhanced interest in, and demand for, convenience, taste, and healthful foods. In the aggregate, food purchases reflect a pattern of excess. Choice relative to individual food products may not yield healthful eating patterns. A compelling public health message that aims for improved dietary patterns and increased physical activity remains a challenge.
- Research and education that helps to clarify the determinants of health and healthful dietary patterns and that supports translation of nutrition sciences into medical education and activities that facilitate transfer of successful nutrition health strategies into public health endeavors and community health strategies will influence long-term national health prospects.

1. INTRODUCTION

For the two out of three adult Americans who do not smoke and do not drink excessively, one personal choice seems to influence long-term health prospects more than any other: what we eat.

From: *Preventive Nutrition: The Comprehensive Guide for Health Professionals, Third Edition*
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Table 1
Leading Causes of Death, 2001

Cause of death	*Number of deaths*
Heart disease	700,142
Cancer	553,768
Cerebrovascular disease	163,538
Chronic lung disease	123,013
Accidents	101,537
Diabetes	71,372
Pneumonia and influenza	62,034
Alzheimer's disease	53,852
Nephritis	39,480
Septicemia	32,238

Adapted from ref. *2*.

This statement, taken from the 1988 *Surgeon General's Report on Nutrition and Health (1)*, offers an indication of both the challenge and the opportunity facing the nutrition community. It is now more than 15 yr since this seminal report, which helped launch a US dietary guidance policy that links dietary choices with prevention of chronic disease. Unfortunately, since then, the weight problems and obesity have worsened, putting millions more at increased risk of death and disease from related chronic conditions. It has become clear that vigorous actions are important to help people eat right, exercise regularly, and take other actions to promote good heath.

This chapter discusses four major points. First, the contribution of the nutrition sciences will assume increasing and compelling importance in the prevention of disease in the 21st century. Second, the greatest challenge to constructive engagement of these issues is dealing with the uncertainty attendant to change. Third, in the face of uncertainty, the role of policy is to bring focus to the points of social and scientific convergence. Finally, in the face of change, the role of the clinician is to bring focus to the individual. The underlying factors and policy implications of these points are discussed in the following sections.

2. FACTORS INFLUENCING NUTRITION AND CHRONIC DISEASE PREVENTION

The factors most likely to influence the nutrition policy agenda of the next century include the national disease profile, demographic profiles, economics, changing meal patterns, changing meal sources, public awareness, professional awareness, the development of scientific insights, and the advent of new technologies.

2.1. National Disease Profile

Fundamentally, any public health agenda is driven to a substantial extent by the population's profile of disease and disability, the nature of the problems at hand, and the rates at which they are changing. The most concrete indicator of the disease profile for Americans is found in the mortality tables. In 2001, approx 2.4 million Americans died. Their death certificates state that the top five causes of death were heart disease (HD), cancer, cerebrovascular disease, chronic lung diseases, and accidents (Table 1). The next

Table 2
Real Causes of Death, 1990

Cause of death	*Number of deaths*
Tobacco	400,000
Diet/inactivity patterns	300,000
Alcohol	100,000
Certain infections	90,000
Toxic agents	60,000
Firearms	35,000
Sexual behavior	30,000
Motor vehicles	25,000
Drug use	20,000

Adapted from ref. *3*.

five most prevalent conditions, listed in order of magnitude, were diabetes, pneumonia and influenza, Alzheimer's disease, nephritis, and septicemia *(2)*.

Currently, 4 of the 10 leading causes of death have important dietary links, including three—HD, cancer, and stroke—that account for almost 70% of all deaths in the United States. As a result of a generation of biomedical research, it is known that diet-related factors, including relationships with physical activity and weight problems, account for a large proportion of these diseases. Studies have variously associated dietary factors or sedentary lifestyles with between one-fifth and one-third of cardiovascular deaths, 20 to 60% of fatal cancers, and 50 to 80% of diabetes mellitus cases, including 30% of diabetes deaths *(3)*. Drawing on available studies to assign responsibility for deaths on the vital statistics ledgers to what we now know to be their root causes reveals just how important dietary factors are to the health of Americans (Table 2). Together, diet and the prevailing pattern of inactivity contribute some 300,000 to 500,000 deaths annually, according them the dubious distinction of either the first or second actual causes of death for Americans, depending on whether one looks to the higher or lower end of the estimated range, and the only factors that are daily factors for the entire population *(3)*.

Moving beyond the mortality tables to the issue of the aggregate burden of preventable conditions (Table 3) is enlightening. Manifest here is the fact that dietary factors predispose, to varying degrees, to many of these conditions, including contributions to the 13 million annual cases of coronary heart disease (CHD), 1.3 million cases of cancer, 700,000 strokes, 17 million cases of diabetes (almost 80% of which are maturity-onset), approx 300,000 low-weight births, and 2500 neural tube defects (NTDs) *(5–9)*. For some of these contributions, solid nutrition-related risk factors have been identified, and discernible progress is already evident for application of the information in prevention and disease control, albeit far short of what should be possible. Some of the most intractable contributions, such as cancers or low birth weight, have nutrition components that are suggestive; however, the mechanisms are less clear. A more extensive rendition of serious health challenges to the American population would also include a number, which is growing rapidly, such as Alzheimer's and arthritis, which are without clearly defined nutritional components. As new understanding is gained about the relationship between dietary patterns and disease outcomes, the role of nutrition is likely to grow as a public policy priority.

Table 3
Annual Burden of Preventable Conditions

Condition	*Annual burden (no. of cases)*
Coronary heart disease	13 million
Certain cancers	1.3 million
Stroke	700,000
Diabetes	17 million
Many injuries	2.6 million hospitalized
Chronic lung disease	28 million
Alcoholic liver disease	14 million alcoholics
HIV and other STDs	16 million
Low weight babies	300,000
Many neural tube defects	2500
Vaccine-preventable diseases	55,000

Adapted from refs. *4–11*.

2.2. Demographic Profiles

Public policy is generally more responsive to groups whose numbers are growing rather than shrinking. Over the last half of the 20th century, the US population increased from 150 million to 294 million in 2004 *(6,12)*. Two other major changes occurred in the demographical characteristics of the US population: the growth of the older population and increased racial and ethnic diversity.

2.2.1. Growth of the Older Population

Life expectancy in the United States reached an all-time high of 77.2 yr in 2001 *(6)*. Over the course of the 20th century, the median age of the population increased by nearly 12 yr, from 23 to 35 yr; the share of the population over age 85 yr grew from 0.2 to 1.5%, and the share of the population under age 25 yr decreased from 54.1 to 35.3% *(13)*. Rapid growth in the older population is expected as the Baby Boomer generation begins to turn age 65 yr in 2011. The nutrition concerns of the elderly are going to move up quickly on the public health agenda. The aging of the US population clearly contributes to the sense of urgency regarding public health.

In the period from 1970 to 2020, the population over age 65 yr will more than double, growing from approx 20 million to approx 54 million (Table 4). In this period, those over age 65 yr will grow from 10 to more than 15% of the population *(14)*. Those over age 85 yr will more than quadruple in that period, growing from 1.5 to almost 7 million by 2020 *(13,15)*. Indeed, if projected to the year 2040, the population over age 85 yr will have nearly tripled again relative to the year 2010 and will then represent 4% of the total population *(15)*.

With the cost of treatments that often accompany aging (not only the coronary bypass procedures, chemotherapy, stroke rehabilitation, and joint replacements that can range from $25,000 to $250,000 each, but also the day-to-day management of problems such as congestive heart failure and diabetes), it is apparent that the aggregate cost of potentially preventable conditions has already become an urgent societal concern that is likely to worsen.

2.2.2. Increasing Racial and Ethnic Diversity

Racial and ethnic diversity began to characterize the US population during the last half of the 20th century, especially since 1980 (Table 5) *(14)*. The aggregate minority

Table 4
Aging of the Population

Year	1900	1920	1940	1960	1980	2000	2020
Number of people age 65 yr or older (in millions)	3.1	4.9	9.0	16.6	25.6	35.0	53.7

Adapted from refs. *13* and *14*.

Table 5
Percent of US Population in Selected Race and Ethnic Origin Groups, All Ages, 1980–2000

Race and ethnic origin	*1980*	*1990*	*2000*
Hispanic or Latino	6.4	9.0	12.5
White	79.9	75.7	69.5
Black	11.5	11.8	12.2
Asian and Pacific Islanders	1.6	2.8	3.9
American Indian and Alaskan Native	0.6	0.7	0.7

Adapted from ref. *14*.

population (people of races other than Caucasian or Hispanic origin) increased by almost 90% between 1980 and 2000, whereas the white, non-Hispanic population grew by about 8% during the 20-yr period. Younger age groups had a higher percentage of minority population than did older age groups. By 2000, the proportion of minority population ranged from 16% for people age 65 yr and older to 39% for those under age 25 yr *(13)*. Changes in the racial and ethnic composition of the population will be reflected in the health of the United States because many measures of disease and disability differ significantly by race and ethnicity.

Policymakers are faced with a challenge in finding successful approaches to improve public health as research continues to evolve and there is too little evidence to know which solutions will be effective and to what extent racial, gender, or just cultural differences determine approaches to success.

2.3. Economics

Economics is a central determinant of public policy. In fact, it is not uncommon for advocacy organizations to define policy strictly in budgetary terms. Nutrition and disease prevention are influenced by several forces of economics, including the economics of eating patterns, which influence the development of disease, and the economics of food production and marketing.

Estimates of the potential effects of diet on the development of chronic diseases have been used to estimate the benefit of improved dietary patterns. Examples from the Food and Drug Administration (FDA) regulations for food labeling and food standards over the past decade are illuminating (Table 6). The economic impact analysis for the Nutrition Labeling and Education Act (NLEA) estimated that the benefits of the amendments to require mandatory food labeling for virtually all foods would result in beneficial changes in food purchases that would be associated with over 39,000 fewer cases of cancer and CHD. The economic benefit of the total NLEA proposals (changes in the information panel, including new nutrient and ingredient information, as well as changes such as new definitions for nutrient content claims and health claims) were

Table 6
Examples of Economic Benefit Estimated in Regulations for Food Labeling and Food Standards

Regulation	*Primary change*	*Economic benefit*
NLEA *(16,17)*	↓Fat and SFA intake (1% of energy) ↓cholesterol intake (0.1% of energy) ↓CHD risk	$4.4–26.5 billion/yr
Folate fortification *(18)*	↑intake folate 0.14 mg/100 g product ↓116 NTDs and 25 infant deaths	$651–788 million/yr
Label *trans* FAs *(19)*	↓*trans* FAs intake. (.04% of energy) ↓LDLC (Method 1) ↓600 heart attacks; 240 fatal	$1 billion/yr
	↓*trans* FAs intake (.04% of energy) ↓LDLC and ↑HDLC (Method 2) ↓1200 heart attacks; 480 fatal	$2 billion/yr

Adapted from refs. *16–19*.

estimated to be $4.4 to $26.5 billion over a 20-yr period *(16,17)*. This analysis was based primarily on an estimated 1% reduction in intake of fat and saturated fatty acids (SFAs) and a 0.1% reduction in consumption of cholesterol *(16,17)*.

Economic impact analyses, prepared for regulations that require fortification of cereal-grain products with folate at 0.14 mg/100 g, also provided estimates of economic benefit. These analyses, based on the occurrence of 2500 cases of NTDs per year, of which a proportion are folate-related, estimated that 116 NTDs and 25 infant deaths would be prevented per year and would result in economic benefits, calculated by the "willingness to pay" methodology to be in the range of $651 to $788 million each year *(18)*.

In 2003, the FDA amended its regulations on nutrition labeling to require that the amount of *trans*fatty acids (FAs) be listed on the Nutrition Facts Food Label on a separate line under the line for the content of SFAs *(19)*. In its economic analysis, the mathematical model used by the FDA showed that reducing the intake of *trans* FAs by 0.04% of energy would reduce low-density lipoprotein (LDL) cholesterol levels and thus CHD risk by about 0.05%, thereby preventing approx 600 heart attacks, including 240 fatal heart attacks. FDA estimates the value of these averted illnesses at approx $2 billion annually ($13.1 billion discounted over 20 yr), factoring in estimates of the statistical value of life, the costs of quality-adjusted life years potentially compromised by illness, and the averted direct medical cost. With slightly different assumptions, the estimates suggest possibly even higher benefits. A mathematical model based on the assumption that *trans* FAs may increase CHD risk by lowering high-density lipoprotein (HDL) cholesterol showed that a reduction in *trans* FAs to 0.04% of energy would reduce LDL cholesterol levels, raise HDL cholesterol levels, lower CHD risk by 0.1%, prevent approx 1200 heart attacks, including 480 fatal heart attacks, valued at approx $2 billion annually ($26.8 billion discounted over 20 yr) *(19)*.

There are no communication models that demonstrate how food labeling might be used more efficiently as an educational tool in control of weight problems and obesity; however,

given the impact that related conditions have on economic costs for the US health care system, ample motivation exists for better use of the food label to make informed choices. It has been estimated that for the US adult population as a whole, overweight- and obesity-related medical spending may account for about 10% of the annual medical expenses and may have reached or exceeded $100 billion. Much of the cost associated with obesity results from adult-onset diabetes, CHD, and hypertension *(20,21)*.

A mathematical model was used to project economic benefit from changing to a healthier diet. This projection estimated that healthier US eating patterns could prevent $71 billion each year in medical costs, lost productivity, and premature deaths associated with CHD, cancer, stroke, or diabetes. Specifically, medical costs account for 47% of the total, premature deaths account for 39%, and lost of productivity associated with morbidity accounts for the remaining 13%. These estimates did not include diet-related costs associated with osteoporosis, hypertension, weight problems, and NTDs *(22)* (*see* Chapter 24).

Another activity that has experienced major support by the federal government is the nutrient references Dietary Reference Intakes (DRIs) adopted by the National Academy of Sciences, Food and Nutrition Board, Institute of Medicine. The DRI initiative began in 1993 *(23)*. The DRIs replace and expand on the Recommended Dietary Allowances (RDAs) that were the acknowledged nutrient standards for the past 50 yr. The DRIs include four reference values: the RDA, the estimated average requirement, the adequate intake, and the tolerable upper intake. Eight reports have resulted from the effort. In these, nutrient group panels reviewed the scientific literature regarding the roles of nutrients in health and disease, interpreted the current data on dietary intakes in North American population groups, and made recommendations for the nutrient or food reviewed. The DRIs provide Americans with guidelines to optimize health and physical functions. At the end of 2003, the Committee on the Use of Dietary Reference Intakes in Nutrition Labeling presented their recommendations on how to use DRIs in food labeling and for discretionary fortification of food in United States and Canada *(24)*. A change to the food label reference values would affect the Daily Values (DVs) used on the food label because nutrient content and health claims in the United States are largely dependent on the DV. Ongoing studies are needed to help determine the effects of new nutrition label reference values and the recommended fortification guidelines.

Another federal initiative, the 1969 White House Conference on Nutrition *(25)*, focused national attention on the need for food assistance programs to address poverty-related hunger and nutrition. These food assistance programs, primarily administered by US Department of Agriculture (USDA), provide different types of food benefits to various target recipients. Three of these programs accounted for 85% of the $38 billion spent on food assistance in 2002: the Food Stamp Program ($20.7 billion), the National School Lunch Program ($6.9 billion), and the Women, Infants, and Children Program ($4.3 billion) *(26)*. The primary intent of the programs is to provide an adequate quantity of food, not to assure that the quality of food choices is optimal for the prevention of chronic diseases. However, the USDA provides reimbursement to states for half of the cost of approved Food Stamp Program education and promotion activities. Thus, there is an opportunity to influence food choices. Also, the USDA's School Meals for Healthy Children regulations have been implemented to ensure that school meals comply with the Dietary Guidelines for Americans, making meals that provide nutrient

adequacy and that are reduced in fat, SFAs, cholesterol, and sodium (Na) available *(27)*. These examples of federal food-related initiatives demonstrate that economic benefits may be achieved by federally mandated dietary change.

Food is an almost $900 billion US industry, and the US food marketing system is the second largest advertiser in the American economy (second to the automotive industry) *(28,29)*. Over 75% of food advertising is communicated via television, showing branded products that consumers can easily identify *(29)*. The highest food advertising expenditures are concentrated on prepared, convenience foods, followed by confectionery items and snacks, alcoholic beverages, soft drinks and bottled water, and cooking products (including sugars and oils). The foods with the highest proportions of advertising expenditures tend to be those that are overconsumed rather than those recommended by US Dietary Guidelines *(29)*. Food manufacturers are responsible for 50% of food advertisements, with food service contributing another 25% and food retailers contributing about 15% *(30)*. Advertising expenditures rose 52% between 1990 and 2000, with the largest increases paid for by food service firms and food retailers *(30)*. The intensity of food advertising reflects several factors. First, food purchases capture about 10% of consumer spending and, hence, provide incentive for competition; second, food is a repeat-purchase industry subject to frequent shifts in consumer opinions; and finally, food is highly branded, with easily communicated images *(29)*.

Recognizing that the food industry is in business to sell food and uses advertising to sell food, advertising can also provide information about healthful food characteristics, appealing to consumers to select healthful food products and thus precipitate competition that shapes the mix of food products in the market. It is in this area that policymakers must continue to get appropriate messages to consumers, influence consumer demand for more healthful products, and provide incentives for the industry to respond to government dietary guidance recommendations.

2.4. Changing Meal Patterns

The forces of change as well as the pace of that change are dramatic for food and nutrition today. Certainly, important changes have occurred in the choices people make. The notion that behavior is immutable was long ago put to rest. From food disappearance data (the foods available for consumption), it is evident that the type of food on the American menu is changing (Table 7). Since 1970, the availability of red meat (mostly beef) is down about one-sixth, fish is up by about one-quarter, and poultry has increased almost twofold. On the other hand, summing the pounds per capita in these three components of the meat group suggests that the combined total poundage available for consumption is up 8%. The largest increase occurred in the period after 1980. Coincidentally, egg availability that had been declining appears to be increasing, a trend that in part reflects consumer use of more prepared foods. Whole milk is down by 70%, whereas milk with lower fat content (<2%) continues to increase in popularity. Per capita consumption of cheese is up almost three times the level it was in 1970 *(31)*. Also, food supply nutrient data show that the total fat and SFAs provided by dairy products have remained constant since 1970 *(32)*. The availability of grain and cereal products is up about 45% and the availability of fresh fruits and vegetables is also up by about 25% *(31)*. Even considering the uncertainty introduced to food disappearance and availability data by waste in prepared foods, it appears that considerable changes have occurred in a relatively short period of time, and many are in the direction of lower SFA/total fat products.

Table 7
Food Consumption (Pounds Per Capita)

	1970	*1980*	*1985*	*1990*	*1995*	*2001*
Red meat	131.9	126.4	124.9	112.2	113.6	111.3
Fish	11.7	12.4	15.0	14.9	14.8	14.7
Poultry	33.8	40.8	45.6	56.2	62.1	66.2
Eggs (#)	308.9	271.1	255.2	234.1	232.3	251.1
Milk, total (gal.)	29.6	25.9	25.0	24.2	22.5	20.3
Milk, whole	24.8	16.5	13.9	10.2	8.3	7.4
Milk, 2% fat	3.2	6.3	7.9	9.1	8.0	7.0
Milk, <2% fat	1.5	3.1	3.2	4.9	6.1	5.9
Cheese	11.4	17.5	22.5	24.6	26.9	30.0
Flour/cereal	135.6	144.7	156.5	180.9	190.2	195.7
Fresh fruits	101.2	104.8	110.6	116.2	122.5	125.8
Fresh vegetables	154.3	151.3	158.4	170.2	180.9	196.6

Adapted from ref. *31*.

Table 8
Food Supply Servings Compared With Food Guide Pyramid

Food group	*Food guide pyramid*	*Food supply servings*
Added fats and oils	Use sparingly	64 g
Added sugars	12 teaspoons	34 teaspoons
Meat, poultry, fish dry beans, eggs, & nuts	2–3 servings	2.5 servings
Dairy products	2–3 servings	1.6 servings
Vegetables	3–5 servings	4 servings
Fruit	2–4 servings	1.4 servings
Grains	6–11 servings	10 servings

Adapted from ref. *33*.

Recently, the USDA developed a method to adjust food supply data for losses and to relate these data to American progress in following the 2000 edition of the Dietary Guidelines for Americans, as depicted in the Food Guide Pyramid (Table 8) *(33)*. These data show that to improve their diets, the population needs to consume more fruits and a greater variety of fruits and vegetables. Three fruits (oranges, apples, and bananas) contribute one-half of the daily fruit intake, and iceberg lettuce, potatoes, and canned tomatoes contribute more than half of vegetable consumption *(33)*. To improve their diets, many people need to change the type of grain and cereals they consume, because whole grains are about one serving per day rather than the minimum three servings that are recommended; cakes, cookies, pastries, and pies account for almost 15% of total grain consumption, with grain-based snack foods (crackers, popcorn, pretzels, and corn chips) accounting for an additional almost 5% *(33)*.

To improve diets regarding the meat group, more selections should be low in SFA and cholesterol, such as legumes and fish. In the dairy group, emphasis should be on low-fat/low-SFA selections because more than half of the daily dairy choices came

Table 9
Food Product Trends, 2003

- Convenient meals and simple solutions; mobile meals and snacks, cookware- and utensil-free; bars and beverages
- Upscale culinary; indulgent, retro, fine dining, American + ethnic
- New forms, varieties, flavored, and colored versions of favorite foods tailored to taste, age, group, and lifestyle
- Healthful food products to prevent or manage disease; avoidance foods—no, less than, and reduced; novel fats; new grain products: organic and natural

Adapted from refs. *35* and *36*.

from cheese and whole milk. Finally, to improve diets, there should be a decrease in added sugars and added fats. The Food Guide Pyramid suggests limiting added sugars to 12 teaspoons a day (based on a calorie level of 2200) rather than the 34 teaspoons provided by the food supply, adjusted for losses *(33)*. For added fats, which provide about 52% of the total fat in the food supply, consuming two-fifths less added fat (approx 25 g)—especially shortening and margarine, lard, beef tallow, butter, and other dairy fats—would help to achieve a total fat intake that is less than 30% of the calorie intake *(33)*. To reach public health goals for a healthier US population, public policy must find approaches to improve healthful food choices and to increase physical activity levels.

As dietary guidance policy has continued to focus on improvements in food choices, there has been a change in the food products available. In 2002, the greatest number of new products introductions occurred since 1995 *(34)*. The confectionery food product category was strongest, followed by bakery, beverages, and sauces and seasonings, and accounted for half of all food product introductions. With convenience vying as a major consideration, consumers, who had grown experienced in restaurant dining, tried new food introductions at home and moved toward classic, retro, and ethnic cuisine, especially those that suggested a gourmet edge (Table 9).

By Fall 2003, the food marketplace reflected additional products that were developed to respond to food labeling changes *(19)* and public health recommendations *(24,37,38)*. The market responded to the new nutrient references (DRIs) adopted by the Food and Nutrition Board, Institute of Medicine, and to earlier recommendations of the Dietary Guidelines 2000 *(37)* and the National Cholesterol Education Program (NCEP) *(38)* by developing an expanded number of products aimed at reductions in blood cholesterol levels—plant stanols and sterols, oat bran, guar gums, and soy proteins; responding to consumer interest to limit sugar intake and addressing diabetes and control of blood sugar levels—sugar replacements and resistant starches; manufacturing products to feature proteins (which have no upper limit in the new DRIs), including whey protein and protein food sources such as egg, fish, gelatin, and yeast; and displaying numerous other food introductions that respond to consumer interest in health, such as those that propose to boost the immune system, promote eye health, and help with digestion.

These food marketing facts suggest a rapid change in what consumers are demanding and purchasing. With more food products that offer taste appeal and convenience and still confusing health claims for foods, the consumer responses is to buy more food.

Despite consumer interest in healthful foods, in the aggregate, food purchases have enhanced the 20th century pattern of excess. The result has been a 15% increase in average daily calories available in the food supply, from 3300 per capita in 1970 to 3800 in 1994 *(32)*. Thus, as the full range of nutritional implications of changes in the food marketplace continues to evolve, the need for a compelling public health message on dietary patterns remains a challenge.

2.5. Changing Meal Sources

Americans no longer purchase all their food as fresh items and commodities to be prepared for meals eaten at home. Consumers still eat most of their main meals at home, and in 2002 restaurant spending grew by only 2% *(35)*. Yet an increasing number of meals are fully or partially prepared outside the home. By 2002, 46% of the $900.1 billion food dollar was spent on food away from home *(28)*. Conventional supermarkets compete with alternative retail outlets including supercenters, warehouse stores, and Internet-based businesses. Competition helps to keep food prices low, and it has been predicted that the first decade of the 21st century will continue a historical decline in food price inflation. The 1970s had average annual food price inflation of 8.4%, the 1980s had 4.6%, and the USDA predicts annual food price inflation of 2.2% for the current decade *(39)*.

The nutritional implications of these trends in meals prepared away from home are not fully determinable. However, an analysis based on national food consumption data calculated that if food away from home had the same average nutritional densities as food at home in 1995, US consumers would have consumed 197 fewer calories per day, reduced their fat intake to 31.5% of calories (rather than 33.6%), and reduced their SFA intake to 10.9% of calories (rather than 11.5%). In addition, the consumption of calcium would have increased by 7% and their consumption of fiber and iron by 9% each *(40)*. Sodium intakes would not have changed significantly, possibly because most sodium is added to food prior to its entry into the household. The preceding data raise concerns, especially for those in certain socioeconomic categories, about the potential of a lower intake of fresh fruits and vegetables and higher caloric—and likely higher fat, especially saturated fat—patterns of consumption, relative to what would be consumed at home. However, time-pressured consumers spent only 10.2% of their 2002 disposable personal income on food, and a broad spectrum of food retail outlets helped to keep prices at low inflation levels *(29)*. Consumers are interested in healthful foods, but health is not yet the overriding consideration regarding which foods to purchase.

2.6. Public Awareness

There is little question that the public is increasingly interested in nutrition and aware of the central issues. In 2002, 70% of consumers responding to a Food Marketing Institute (FMI) survey said that their diets could be healthier and that they could ensure a healthy diet. About 86% agreed and 53% strongly agreed that in most cases, eating healthfully is a better way to manage illness than medication, up from 76 and 43%, respectively, in 2001 *(41)*. The eating for health concept is also shown in the 2002 data, which found that 72% of consumers almost always/sometimes look at health claims, up 6% over the previous year *(41)*. Similarly, in the 1999 Discovery Health Survey, 66% of respondents knew that saturated fat was related to disease *(42)*.

However, recent FMI survey data revealed interesting findings relative to the top three consumer concerns about the nutritional content of foods in 2003 compared with 1996: fat content (49%, down from 60%); sugar content (18%, up from 12%); and salt content (18%, down from 28%) *(43)*. The survey information does not provide information to explain the change nor to relate the response to food purchase behavior. Such information could be useful to policymakers who offer dietary guidance recommendations.

Additional data that show consumer interest in food and health is found in 2002 data, in which most or many consumers reported that they were very interested in trying health-promoting foods (54%) and wanted products designed to help them with lowering blood cholesterol levels and preventing cancer (54%) and to help with blood pressure and diabetes (51%), osteoporosis (49%), and weight control (49%) *(44)*. These data support consumer health motivations in selection of food products.

Americans also like to eat. In 2002, survey data showed that 72% of consumers agreed and 30% strongly agreed with the statement, "I eat foods that I enjoy even if they are not good for me"—up from 64 and 24%, respectively, in 2001. Also, 34% strongly agreed and 14% agreed with the statement, "I eat what I want and don't think much about how it affects my health"—up from 25 and 9%, respectively, in 2001 *(43)*. This suggests that consumers may compartmentalize their information; they have awareness and knowledge of food/nutrient and disease relationships but make choices relative to individual products that may not translate into a healthful eating pattern. Policymakers and other leading health groups must be vigilant to recognize that consumers are likely to seek simple solutions for health achievement such as those suggested by new food products and even the newest nutrition research report. Instead, the media, nutrition scientists, and policymakers need to develop an engaging and consistent communication strategy to put the latest nutrition research and the newest food product innovations in the context of existing knowledge about dietary patterns and health.

The task is complex because in addition to taking responsibility for providing contextual information, a major challenge must be faced in helping people sort out the central tendencies of new information released to the public from scientific research—in effect, helping people understand what's important for them, given their particular circumstances.

2.7. Professional Awareness

In the last decade, there has been a concerted effort to increase physician awareness and interest in nutritional issues. There is evidence that physicians are aware and use the treatment guidelines of the NCEP (ref. *38*) and of the National High Blood Pressure Education Program (ref. *45*). In addition, health professionals have a growing concern about weight problems and obesity and appropriate health messages to address this public health priority *(21)*.

Is the health professional community doing enough? Despite a number of public and private sector efforts aimed at reducing obesity, only modest success is suggested and no success is evident in reversing the trend in the increasing prevalence of weight problems and obesity. In the most recent national data (1999–2000), nearly two-thirds of US adults were overweight or obese, with twice as many overweight children and three times as many overweight adolescents as there were in 1980 *(46,47)*.

From 1999 to 2000, under 20% of US adults had high serum cholesterol levels (at or above 240 mg/dL). The age-adjusted mean serum cholesterol level was 204 mg/dL, statistically unchanged from 205 in 1988–1994 *(6,48)*. Those with high serum total cholesterol

who also have low levels of HDL-C may be at greater risk of CHD than others. The data showed that no age, gender, or race/ethnicity group had more than 11% of its population with this lipid combination *(49)*. The age-adjusted prevalence of participants reporting current use of cholesterol-lowering medications rose from 3.1% in 1988–1991 to 7.9% in 1999–2000 *(50)*.

Current rates of hypertension control, although improving, continue to be low. In 1999–2000, 28.7% of National Health and Nutrition Examination Survey participants had hypertension (140/90 mmHg or greater), an increase of 3.7% from that in 1988–1991. In the latest data, 68.9% were aware of their hypertension, 58.4% were treated, and hypertension was controlled in 31% *(50)*.

An editorial written to accompany the release of the 2003 national guidelines on blood pressure noted that successful prevention of hypertension requires the will to act by clinicians, health care system administrators, purchasers of health care, patients, and communities through a comprehensive program of lifestyle, environmental development, and clinical intervention *(51)*. Also, by extension, it could be said that the same commitment is needed to prevent and control high cholesterol levels and to control rates of excessive weight and obesity.

Health care provider attitudes have changed substantially in recent years, but nutrition education in medical schools is still weak, particularly when considered against the translational challenge physicians will face in the future (*see* Chapter 36). Public policy can do much to turn the improvements in health professional awareness of the relationship between nutrition and health into positive influences on the health of the public; however, if the full potential is to be reached, then a close partnership will be required with the scientific and health provider communities.

2.8. Development of Scientific Insights Coupled With New Technologies

Clearly, new scientific insights can change the role of nutrition in prevention, especially the public policy aimed at chronic disease prevention. A good example is what has already happened with the NCEP based on more than half a century of research about the relationship between blood lipids and cardiovascular disease.

The future will undoubtedly hold much more as the nutritional sciences incorporate the lessons from molecular biology and genetics. The priority categories of the Federal Interagency Committee on Human Nutrition Research indicate that we may expect advances in these areas: nutrient requirements throughout the life cycle; nutrient interactions and bioavailability; nutrition and chronic diseases; energy regulation, obesity, and eating disorders; nutrition surveillance and monitoring methodology; and nutrition education techniques.

Impressive change is also occurring in the science and technology base. These advances have contributed to important developments in the epidemiology of nutrition and chronic diseases, in the understanding of nutrient action at the molecular level, and about individual variation in response to nutrient challenges. Perhaps the most dramatic developments, if only in terms of the pace of change, are coming from the increasing understanding of how people vary.

The human genome project has made a large difference regarding expectations for nutrition and prevention in a short time. The year 2003 marked the 50th anniversary of the discovery of the double-helical structure of DNA. The Human Genome Project (HGP) began in 1990, and all of its initial projects have now been achieved at least 2 yr ahead of

expectations *(52)*. In 2003, scientists at the HGP wrote that the project's new research strategies and experimental technologies and large-scale generation of community resource data sets had introduced new dimensions into biological and biomedical research. Advances in genetics, comparative genomics, biochemistry, and bioinformatics have provided scientists with an improved repertoire of research tools to analyze and understand at an unprecedented level of molecular detail. In describing the challenges that lie ahead, a particular relevance to nutrition is cited in Challenge II-6: to develop genome-based tools that improve the health of all. Population differences in allele frequencies for some disease-associated variants could be a contributory factor to health disparities; therefore, incorporating this information into preventive and/or public health strategies would be beneficial. Furthermore, research can explore the relationship between genomics and health disparities by evaluating the diverse contributions of socioeconomic status, culture, discrimination, health behaviors, diet, environmental factors, and genetics *(52)*.

In the development of new food products, engineering genetic change is one of several technologies used to change plant components, including fatty acid composition and levels of selected amino acids and vitamins and of antioxidants such as polyphenols. One development in recent years that offers a range of food products with various sought-after properties and is now in the marketplace is structured lipids. Structured lipids are tailor-made fats and oils designed to have improved nutritional or physical properties because of modifications to incorporate new fatty acids or to change the position of existing fatty acids on the glycerol backbone by chemically and/or enzymatically catalyzed reactions and/or genetic engineering *(53)*. Several of these processes have resulted in fats with low *trans* FA contents. Genetic engineering has allowed the development of seed oils (canola, soybean, and sunflower) with increased ratios of high-oleic, monounsaturated FAs to linoleic, polyunsaturated FA and/or with somewhat increased levels of SFAs. The resulting oils can eliminate the need for hydrogenation and, therefore, the production of *trans* FAs. Transesterification of seed oils by enzymes (e.g., lipases) and chemical esterification has been used in the production of *trans*-free margarines *(53)*. Esterification of seed oils either chemically or with enzymes also has been used to produce reduced-calorie fats (approx 5 kcal/g) and fat substitutes. In the latter, most of these structured lipid products contain fatty acids that are not naturally present in edible oils and fats and lack important essential FAs, but match the chemistry and functions of natural fats. Although the field of structured lipids is relatively new, consumers are likely to purchase products that offer characteristics such as a reduced content of calories and *trans* FAs.

3. POLICY IMPLICATIONS

In the face of uncertainty, the role of policy is to bring focus to the points of social convergence. Policy is fundamentally targeted to wielding levers that address common denominators.In saying nearly 150 yr ago that "politics is nothing but medicine on a larger scale" *(54),* Virchow could have had the challenge of dietary guidance in mind. In this matter of great importance to the health of the American people, the task is reminiscent of the basic challenge of any policy process: the task of finding the right balance in the dialectic of the debate between the dictum "the greatest good for the greatest number" and the Hippocratic admonition "first, do no harm."

A convergence is emerging from the often disparate vantage points of studies of different disease processes in the American population. From studies of CHD etiology comes the lesson that intake levels of SFAs, TFAs, and cholesterol correlate with higher

Table 10
Preliminary Dietary Guidelines for Americans, 2005

- Consume a variety of foods within and among the basic food groups while staying within energy needs.
- Control calorie intake to manage body weight.
- Be physically active every day.
- Increase daily intake of fruits and vegetables, whole grains, and nonfat or low-fat milk and milk products.
- Choose fats wisely for good health.
- Choose carbohydrates wisely for good health.
- Choose and prepare foods with little salt.
- If you drink alcoholic beverages, do so in moderation.
- Keep food safe to eat.

Adapted from ref. *57*.

serum cholesterol levels and, therefore, higher risk of CHD *(55)*. From studies of cancers of the breast, colon, and prostate, indications are offered of the increased risk attendant to higher consumption patterns of animal fat and total energy *(56)*. From studies of diabetes and obesity come the insight that Americans' dietary patterns are characterized by portions that are too large and too high in sugars and carbohydrates low in fiber. This consensus is one of several reflected in the policy statement known as the *Dietary Guidelines for Americans* (ref. *57*; Table 10). How well do the *Dietary Guidelines for Americans* serve their audience, especially US consumers? Perhaps reasonably well; however, policymakers need to consider improvements in the revised edition of the guidelines (which is scheduled for release in 2005) to better attain the goals of increased physical activity and healthful dietary patterns without excessive consumption.

There is now national attention and concern regarding the fact that the population is too sedentary and that both consumption and food supply data indicate calories are up from a decade ago. Currently in the United States, food technologies are advancing as quickly and sometimes in advance of human nutrition research and scientific consensus, and consumers like the idea that a food product can promise to improve a broad (and complex) health factor such as the immune system.

Challenges remain, first to interpret the latest diet and health data and then to translate the specific, lengthy, and complex information into simple messages via the Dietary Guidelines, the Food Guide Pyramid (or its graphic replacement), and the food label and to collaborate in understanding how to influence Americans to achieve healthful eating patterns and maintain daily moderately vigorous physical activity.

As we progress into the 21st century, the issue of dietary supplements presents a challenge for dietary guidance from the perspective of several disease conditions. With an aging population, an emerging science based on the importance of foods relatively higher in certain micronutrients, and a population that seems already predisposed to the use of dietary supplements, the issue of supplements will have to be more constructively engaged in any discussion of dietary guidance. Indeed, with increased discretionary fortification of foods *(24)*, it is becoming futile to dichotomize policy discussions and dietary guidance advice between whole foods and dietary supplements (*see* Chapter 4).

Of course, there are other components to nutrition policy beyond those of dietary guidance. Some examples of a few ways that public policy can better capture the

Table 11
Public Health Nutrition Interventions

Nutrition goals
Dietary guidelines
Food assistance
Food safety regulation
Food labeling
Tax policies
Linkage to other sectors

convergence in perspectives for the benefit of Americans are shown in Table 11. The importance of the commitment to improve dietary patterns can be demonstrated by policy decisions that would help in shaping the economic supports and incentives built into food assistance programs; aiding producer groups; emphasizing more healthful and appropriate amounts of food; supporting physical environments in schools, the workplaces, and communities; and supporting sustained marketing of the agreed upon principles. The current demographic and dietary trends transform these issues from "nice things to do" to those of "social imperatives." Action is compelled, even in the face of uncertainty.

3.1. Clinical Implications

The role of the clinician and other health care providers is to bring focus to the points of individual variation. Most policy processes are geared to the points of convergence, but the points of individual variation must also be addressed, and they will be increasingly prominent in the coming years as the science unfolds. It ultimately falls to the clinician to aid patients in the considerable challenges of sorting through what they perceive as conflicting advice, developing strategies for applying general guidance to their particular living circumstances, and identifying ways in which such guidance parts company with the character of their personal levels of risk. Often, these are likely to be complex issues requiring more sophisticated information.

3.2. Summary and Recommendations

At this point, the community of health professionals is ill-equipped to respond appropriately to this obligation. More medical schools need what might be reasonably termed a *clinical nutrition curriculum* (*see* Chapter 36) The initiative of the National Institutes of Health aims to help translate nutrition sciences into medical education with the Nutrition Academic Award Program *(58)*. In the next several years, it will be useful to assess the Program's outcomes and to identify the successful strategies that were implemented to overcome barriers in training physicians in human nutrition and to foster collaborative and dissemination efforts with other medical schools as well as other health care professional schools.

Physicians have lagged behind the general public in acknowledging the relationship between nutrition and health. The solution to such shortfalls can only come with improvements in the nutritional component of educational programs for medical, nursing, and other health care providers; changes in the structure of health services delivery to allow for competent dietary interventions; and introduction of economic incentives to ensure that those interventions are delivered. The fact that more than 76 million

Americans are already enrolled in managed care settings and that the number continues to grow exponentially holds open the prospects for such changes but only if a concerted and coordinated effort works to ensure that they occur *(6)*.

Futurists tell us that the years ahead will be dazzling. The results of advances in the research base could have major implications for the kinds of nutrition targets that will be set in the 21st century. As noted 50 yr ago by Dr. W. Henry Sebrell, an early director of the National Institutes of Health *(59)*:

> *By many indications, this country's major needs in nutrition today are as follows: (1) control of obesity, (2) elucidation of the role of nutrition in the chronic diseases, (3) assessment of nutritional status as a step toward control of border line deficiencies, (4) means of complete intravenous alimentation, and (5) additional knowledge regarding nutrition in the aged, those under stress, and in the convalescent.*

The measure of our success will be found in the extent to which we have been able to change this agenda some 50 yr later.

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II Cancer Prevention

2 Prevention of Cancers of the Esophagus and Stomach

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KEY POINTS

- Prevention of both tobacco use and heavy alcohol consumption are key factors in primary prevention of cancer of the upper gastrointestinal (GI) tract.
- Consumption of fruits and vegetables is lower than optimal in the United States, and increased consumption of these foods has beneficial effects on the prevention of esophageal and stomach cancers as well as other major chronic diseases.
- Specific micronutrients or combinations of micronutrients may be effective in the prevention of upper GI tract cancers, but this has not yet been firmly established in randomized controlled trials.
- *Helicobacter pylori* is a gastric carcinogen. A relatively small proportion of persons infected with *H. pylori* are ultimately diagnosed with gastric cancer; therefore, an understanding of the cofactors in this process is an important area of research.
- Barrett's esophagus is believed to be a premalignant lesion for esophageal adenocarcinoma. Obesity, as determined by elevated body mass index, is also associated with adenocarcinoma of the esophagus, the rates of which are increasing in the United States.

1. INTRODUCTION

This chapter focuses on lifestyle factors that are associated with cancers of the esophagus and stomach. Unlike such major cancers as prostate and breast, whose etiologies presently remain obscure (hindering primary prevention), cancers of the upper gastrointestinal (GI) tract offer well-defined intervention opportunities. Epidemiological studies have clearly established the important role of alcohol, tobacco, and diet, and recent findings have documented the relationship between infection with *Helicobacter pylori* and cancers of the upper GI tract. These factors and their interactions are discussed for cancers of each of these two sites, which together account for approx 35,000 new cases and 26,000 deaths annually in the United States *(1)*.

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2. CANCER OF THE ESOPHAGUS

For many years, cancer of the esophagus in the United States, and in most areas worldwide, was virtually synonymous with squamous cell carcinoma *(2)*. Therefore, most of the established risk factors for esophageal cancer are specific to this cell type, which comprised the vast majority of cases in studies of this cancer. Recent shifts in the histopathological cell type have given rise to a rapid increase in the incidence of adenocarcinoma of the esophagus in the United States, particularly among white males *(3,4)*. Because of the increasing importance of esophageal adenocarcinoma, a separate section discusses this entity, which may differ in etiology from squamous cell carcinoma.

2.1. Squamous Cell Carcinoma

2.1.1. Tobacco and Alcohol Consumption

Both tobacco and heavy alcohol consumption are well-established risk factors for esophageal carcinoma for both men and women. In the United States and other Western countries, more than 90% of the risk can be attributed to the individual and joint effects of tobacco and alcohol *(5)*.

An early study by Wynder and Bross *(6)* graphically examined the interaction between alcohol and tobacco, and the data suggested a multiplicative effect. Tuyns et al. *(7)* more formally evaluated this relationship in data from a case–control study in Brittany. At the highest level of consumption of both alcohol (≥121 g of ethanol per day) and tobacco (≥30 g/d), the relative risk of esophageal cancer was 156 compared to that of non- or light consumers. The increased risk associated with alcohol consumption appears exponential, whereas increased tobacco smoking appears to yield a more linear increase. Saracci *(8)* estimated that the excess risk because of the interaction of alcohol and tobacco is about 25-fold.

Data from a case–control study in Italy are presented in Table 1 *(9)*. Study subjects included 271 male cases and 1754 male controls with acute illnesses unrelated to tobacco and alcohol consumption. Even with a reference category that included moderate alcohol consumption (<35 drinks/wk) by nonsmokers, the estimated relative risk of esophageal cancer among heavy smokers (≥25 cigarettes/d for 40 yr or more) and very heavy drinkers (≥60 drinks/wk) was 22. In 1994, this report was updated to include women *(10)*. Among alcohol drinkers (any vs none), similar risks were observed for women and men (3.0 and 4.7, respectively); however, male abstainers had a twofold increased risk, whereas female nondrinkers had a reduced risk (0.7) compared with light-to-moderate drinkers. This study of esophageal cancer failed to support the hypothesis posed by Blume *(11)* that women may be more susceptible to the effects of alcohol—at least for this particular cancer site. A recent analysis pooled data from three case–control studies in the provinces of Milan and Pordenone (1984–1993) *(12)*, the provinces of Padua and Pordenone and the greater Milan area in northern Italy (1992–1997) *(13)*, and in the Swiss Canton of Vaud (1992–1999) *(14)*. The studies examined 114 female cases of squamous cell esophageal cancer and 425 female controls and concluded that,similarly to men in northern Italy and Switzerland, tobacco smoking and alcohol drinking are the most important risk factors for this neoplasm in women in these regions *(15)*. An additive interaction (odds ratio [OR] = 12.75) was observed for current smokers with heavy drinking more than three drinks a day).

A Japanese study by Hanaoka et al. *(16)* and a study in northern Italy by Zambon et al. *(17)* recently examined whether the increased risk of esophageal cancer attributed to

Table 1
Adjusted Odds Ratios[a] for Cancer of the Esophagus by Alcohol and Tobacco Consumption

	Alcohol (drinks per week)		
Smoking status	*<35*	*35–59*	*60+*
Nonsmoker	1.0[b]	2.2	2.6
Light	2.1	4.4	5.5
Moderate	4.4	9.7	11.4
Heavy	8.4	18.5	21.8

[a]Adjusted for age, residence, education, and profession.
[b]Reference category.
(Adapted from ref. *9*.)

alcohol use is a function of the dose of ethanol or whether the type of alcoholic beverage and its other constituents play a role in esophageal cancer. Their findings confirmed those of others, which indicated that the amount of alcohol consumed, rather than any particular type, is the primary determinant of risk.

The relationship between the role of tar yield of cigarettes and esophageal cancer has only been recently examined. The tar-yield of cigarettes has decreased over the last few decades *(18)*, which may have influenced the recent trend of declining mortality from upper digestive tract neoplasms in males in several industrialized European countries *(19,20)*. An earlier study in northern Italy reported an odds ratio of 2.3 for esophageal cancer in high-tar cigarette smokers compared with low-tar smokers *(21,22)*. The recent analysis of results from two case–control studies (from Italy and Switzerland) that involved 395 squamous cell esophageal cancer cases and 1066 matched controls concluded that there is a direct relationship between tar yield of cigarettes and esophageal cancer. The odds ratios for current smokers compared to those who had never smoked were 4.8 and 5.4 for less than 20 mg and greater than 20 mg of tar, respectively, for esophageal cancer based on the brand of cigarette smoked for the longest time *(23)*.

2.1.2. Thermal Irritation

Thermal injury as a result of drinking very hot liquids has been suggested to increase the risk of esophageal cancer by increasing susceptibility to other carcinogenic exposures *(24–26)*. This hypothesis has received some support from both ecological and analytical studies. Persons living in regions of the world that have high rates of esophageal cancer, such as northern Iran and Siberia, are reported to drink excessively hot tea *(27,28)*.

In Puerto Rico, Martinez *(29)* found that more cases than controls reported drinking hot, rather than warm or cold, coffee. Both Segi *(30)* and Hirayama *(31)* found an increased risk of esophageal cancer in persons consuming hot tea gruel. In Latin America, several studies have examined the role of maté drinking. DeStefani et al. *(32)* found a strong association between hot maté consumption and risk of esophageal cancer in Uruguay. An earlier case–control study in Brazil failed to find a significant association *(33)*. In 1994, Castelletto et al. *(34)* examined the role of maté in an Argentinean case–control study. They found alcohol, tobacco, and barbecued meat—but not hot maté—to be the primary risk factors. However, hot maté consumption was found to be significantly associated with precancerous lesions of the esophagus in a cross-sectional endoscopic survey in southern Brazil *(35)*.

A study of chronic esophagitis, a precursor lesion for esophageal cancer, in a high-risk region in China lended support to an etiological role of thermal injury *(36)*. A greater than fourfold excess of mild and moderate esophagitis was found in young persons ages 15 to 26 yr who consumed burning hot beverages (OR = 4.39, 95% confidence interval [CI] 1.72–11.3). This study design minimized recall/response bias because case–control status was not known at the time of interview and suggests that this factor may be important at a relatively early stage in the development of this cancer.

2.1.3. Nutrition

2.1.3.1. Dietary Studies. In studies worldwide, fruits and fresh vegetables are consistently associated with decreased risk of esophageal cancer, even after controlling for tobacco and alcohol use. Deficiencies of vitamin C, one of several micronutrients contained in fruits and vegetables, have been reported in several areas of the world that have exceptionally high rates of esophageal cancer. These include northern Iran *(27)*, Linxian County, China *(37)*, northern and eastern Siberia *(28)*, and Hungary *(38)*, among others. Other dietary deficiencies are also strongly associated with esophageal cancer risk; these include iron, riboflavin, niacin, molybdenium, zinc, and other trace elements *(39,40)*.

The 1961 report by Wynder and Bross *(6)* noted significantly lower consumption levels of green and yellow vegetables among male cases compared to controls as well as a nonsignificantly lower consumption level of fruit. A case–control study in Singapore determined that potatoes (relative risk [RR] = 0.4; $p < 0.05$) and bananas (RR = 0.3; $p < 0.01$) are protective *(41)*. Frequent consumption of 16 different fruits and vegetables was associated with decreased risk of esophageal cancer in Iran *(42)*. Relative risks for high versus low consumption levels ranged from 0.4 to 0.9, and findings for 10 of the 16 foods were significantly protective. Similar findings were reported in a case–control study in northern Italy *(43)*. High consumption of raw vegetables, citrus fruit, and other fruits were inversely associated with esophageal cancer, with odds ratios of 0.3, 0.4, and 0.5, respectively, after accounting for smoking and drinking.

A significant inverse trend ($p < 0.001$) was reported between monthly vitamin C consumption and esophageal cancer in white males in New York State *(39)*. A weaker but significant inverse association was observed for vitamin A intake ($p = 0.03$). A fivefold reduction in risk was also found in the highest tertile of fruit and vegetable consumption (>81 times/mo). A report from New York found no association with vitamin C that was derived from vegetables *(44)*. However, in this study, only 24% of the eligible cases were included, and they may not be representative of the total series of cases.

Ziegler et al. *(45)* found significant inverse associations between relative risk of esophageal cancer and five indicators of general nutritional status, including total fruit and vegetable consumption (RR = 0.5; p-trend < 0.05). This case–control study focused on high-risk African-American males in Washington, DC. An index of vitamin C intake yielded an estimated relative risk of 0.55 (p-trend < 0.05) for the highest tertile of consumption. The only other micronutrient significantly inversely associated with risk was riboflavin.

Two case–control studies conducted in the high-risk region of Calvados, France, demonstrated a protective effect of vitamin C on esophageal cancer risk *(46,47)*. Approximately threefold significant reductions in risk were observed at the highest level of intake of citrus fruits and dietary vitamin C. Similarly, DeCarli et al. *(48)*

reported a relative risk of 0.3 (95% CI 0.1–0.6) for high-level fruit consumption and nonsignificant reductions in risk for high-level vegetable intake. In India, Notani and Jayant *(49)* found a more modest reduction from high-level fruit intake (RR = 0.8, 95% CI 0.5–1.3) but a significant risk reduction among daily consumers of vegetables (RR = 0.4, 95% CI 0.2–0.7).

Two 1988 reports supported the findings of others that were indicative of protection from high intake of dietary vitamin C and fresh fruits *(50,51)*. Brown et al. *(50)* found a significant halving in risk in the highest tertile of consumption of citrus fruit, all fruits combined, and dietary vitamin C ($p < 0.05$). A study conducted in California by Yu et al. *(51)* showed a relative risk of 0.4 (95% CI 0.2–0.8) for high-level consumption of raw vegetables and fresh fruit. Li et al. *(52)* found no reduction in esophageal cancer risk associated with fruit consumption in a high-risk region of China; however, a homogeneously low level of intake of fruit in this population makes it a poor one in which to evaluate the association *(53)*. In a large case–control study in Hong Kong, Cheng et al. *(54)* reported strong protective effects (p-trend < 0.001) associated with consumption of citrus fruits and other fruits. The proportion of esophageal cancer cases attributable to low-consumption levels of citrus fruits in this population was estimated to be 26%. A retrospective cohort study of esophageal cancer in Linxian, China, reported a significant reduction in risk associated with regular consumption of fresh vegetables (RR = 0.66, 95% CI 0.44–0.99) *(55)*.

A large Italian study of esophageal cancer in lifelong nonsmokers afforded the opportunity to evaluate other risk factors in the absence of residual confounding by tobacco use *(56)*. Although the major risk factor was not unexpectedly alcohol, green vegetables and fresh fruit were associated with significantly reduced relative risks of 0.6 and 0.3, respectively. Similar reductions in risk were associated with β-carotene intake. The estimated relative risk for the combination of high alcohol and low β-carotene was 8.6, with an attributable risk of approx 45%.

In addition to fruits and vegetables and their constituent micronutrients, several other dietary factors have been proposed as candidate protective factors, although the epidemiological evidence is currently considerably more limited. One such factor is green tea, Camellia sinensis (*see* Chapter 3). Experimental studies have demonstrated antimutagenic and anticarcinogenic effects, especially in the esophagus *(57–60)*. Findings in a recent population-based, case–control study in China provided some support to this hypothesis *(61)*. After adjustment for confounders, including tobacco and alcohol, a significant halving of risk was observed in women drinking green tea (OR = 0.50, 95% CI 0.30–0.83), and an inverse dose–response was observed. The findings in men were not statistically significant; however, a significant protective effect was observed in both men and women who did not smoke or drink alcohol. Because green tea, as well as other drinks, can be consumed at hot temperatures, and because excessively hot fluids have been associated with increased risk of this cancer, the relationship between drinking burning-hot fluids was also evaluated. The protective effect of green tea was limited to tea taken at normal temperatures. A more recent case–control study by Ke et al. *(62)*, which involved 1248 cases and the same number of controls in a high-risk region for esophageal cancer in south China, examined the relationship between esophageal cancer and drinking Congou tea, a type of black tea grown in China. An inverse association was observed between consumption of Congou tea (OR = 0.4, 95% CI 0.28–0.57) and a strong dose–response relationship ($p < 0.001$) after adjusting for alcohol drinking

and smoking. The reduced risk was more pronounced if Congou tea was consumed hot (OR = 0.04, 95% CI 0.01–0.13) rather than cold.

Ginseng, which may be taken as a tea, powder, or as a slice of the root, has also been proposed as a potential anticarcinogen. Unlike the polyphenols in green tea, no specific component or mechanism has been elaborated *(63,64)*. Yun and Choi *(64)* reported a case–control study in Korea, where ginseng is commonly used. The relative risk of esophageal cancer associated with ginseng intake was 0.20 (95% CI 0.09–0.38) after adjustment for tobacco, alcohol, and other confounders. This large reduction in risk was observed in both smokers and nonsmokers. Additional studies are obviously necessary to confirm this preliminary finding.

2.1.3.2. Biochemical Studies. Numerous studies have examined biochemical nutritional indicators in blood or tissue, with a particular focus on antioxidants. Chen et al. *(65)* collected blood specimens from a sample of the population in 65 different counties in China and correlated the concentration of more than 10 different antioxidants with county-specific mortality rates for several cancers, including esophageal cancer. A highly significant inverse relationship was found between esophageal cancer rates and both plasma ascorbic acid and selenium in men and selenium in women. Another study in a high-risk region of China found low levels of zinc *(66)*. A recent population-based, case–control study conducted in Washington *(67)* found no significant difference in nail zinc concentrations between esophageal cancer cases and controls but found a large and significant reduction in risk associated with dietary intake of zinc from foods and supplements (ORs of 0.5 and 0.1 for the middle and upper tertile of consumption, respectively; p-trend < 0.001). Other elements in nail tissue associated with esophageal cancer were iron (OR = 2.9 for high vs low levels), calcium (OR = 2.6), and cobalt (OR = 1.9). Although this study suggests a number of differences in mineral levels of cases and controls that reflect differences in intake, metabolism, or both, additional investigation is warranted to determine which, if any, of these findings is etiologically meaningful.

2.1.3.3. Chemoprevention Studies. Chemoprevention, as defined by Sporn and Newton *(68)*, is prevention of cancer with pharmacological agents used to inhibit or reverse the process of carcinogenesis. In this relatively new field, which has grown in acceptance since the 1980s, esophageal cancer is one of the first cancer sites for which results from completed trials are available.

In 1985, Munoz et al. *(69)* reported findings from the first short-term intervention trial. A total of 610 subjects ages 35 to 64 yr in the high-risk region of Huixian, China, were randomized to receive 15 mg (50,000 IU) of retinol, 200 mg of riboflavin, and 50 mg of zinc or placebo once per week for 13.5 mo. A total of 567 participants completed the trial and underwent endoscopy for histological diagnosis of premalignant lesions of the esophagus (esophagitis, atrophy, dysplasia). The combined treatment had no effect on the prevalence of precancerous lesions of the esophagus. However, it should be noted that the dose was relatively small and the intervention period was short. Micronuclei in exfoliated cells of buccal and esophageal mucosa were evaluated in 170 study subjects from this same trial as an indicator of chromosomal damage *(70)*. No reduction in the prevalence of micronuclei was found in buccal mucosa of subjects after treatment, but a significant reduction in the percentage of micronucleated cells in esophageal mucosa was observed in treated subjects (0.19%) compared to the placebo group (0.31%; $p = 0.04$). In a third report from this same trial, Wahrendorf et al. *(71)* re-analyzed data by blood levels of retinol, riboflavin, and zinc at the beginning and end

of the trial, because improvement in blood retinol and zinc levels had been observed in the placebo group as well as the actively treated group. Individuals who had large increases in retinol, riboflavin, and zinc blood levels were more likely to have a histologically normal esophagus at the end of the trial, regardless of treatment group.

Two large intervention studies conducted in the high-risk population of Linxian were reported in the mid-1990s *(72,73)*. A 6-yr randomized trial of daily vitamin/mineral supplementation versus placebo found no significant reductions in cancer incidence or mortality among adults with pre-existing precancerous lesions of the esophagus *(72,74)*. The larger trial in this area included 29,584 subjects from the general population who were randomly allocated to combinations of retinol and zinc; riboflavin and niacin; vitamin C and molybdenum; and/or β-carotene, vitamin E, and selenium in doses of one to two times the US Recommended Daily Allowances (RDAs). Significantly reduced total mortality (RR = 0.91, 95% CI 0.84–0.99) and stomach cancer mortality (RR = 0.79, 95% CI 0.64–0.99) were observed in those taking β-carotene, vitamin E, and selenium. No significant effects on mortality or cancer incidence, including esophageal cancer, were observed for any of the other vitamin/mineral combinations.

Wang et al. *(75)* evaluated whether any of the vitamin/mineral supplement combinations affected the prevalence of clinically silent precancerous lesions and early invasive cancers of the esophagus and stomach, as determined by endoscopy and biopsy in this same trial. No significant reductions in risk of dysplasia or cancer were observed for any of the supplements, although retinol and zinc were suggestively associated with a lower risk of gastric cancer (OR = 0.38; $p = 0.09$). Similarly, Dawsey et al. *(76)* evaluated the effect of the single vitamin/mineral supplement used in the trial of persons with esophageal dysplasia to determine if treatment reduced the prevalence of histological dysplasia or early cancer of the esophagus or gastric cardia. Modest, nonsignificant risk reductions were observed compared to placebo (OR = 0.86, 95% CI 0.54–1.38). The authors concluded that longer interventions with larger numbers of subjects are required to adequately evaluate the effectiveness of micronutrient supplementation in this high-risk population. In subjects from the same trial, Rao et al. *(77)* evaluated whether epithelial proliferation, an early step in carcinogenesis, was reduced by treatment after 30 mo of intervention. The results were similarly inconclusive.

A total of 778 subjects from the Linxian and Huixian China studies were followed with linear repeated biopsies and histopathology examination for 11 yr (1989–2000). Of these, 400 subjects with different severity of esophageal precancerous lesions were randomly divided into two groups for intervention studies in which either calcium , decaffeinated green tea, or placebo were administered daily for approx 1 yr *(78)*. The study concluded that there was no beneficial effect from either calcium or decaffeinated green tea in alleviating esophageal precancerous lesions and abnormal cell proliferation patterns.

2.2. Adenocarcinoma

2.2.1. Barrett's Esophagus and Medications

Barrett's esophagus is characterized by the replacement of the lower esophagus, which is normally stratified squamous epithelium, by metaplastic columnar epithelium *(79)*. This condition, attributed to chronic esophageal reflux, is believed to be a premalignant lesion for esophageal adenocarcinoma *(80)*.

Similarly to esophageal adenocarcinoma, Barrett's esophagus displays a similar age, race, and gender distribution: it is most common in Caucasian males over age 40 yr *(3,81)*.

The reported incidence of esophageal adenocarcinoma in patients with Barrett's is from 30 to more than 100 times greater than the rate observed in the general population *(81–84)*.

There also appears to be a familial form of this disease, inherited as an autosomal dominant trait *(85–87)*. Recent reports of families with the inherited form of Barrett's provided additional support for Barrett's as a precursor lesion *(85,87)*.

A related hypothesis has proposed that the use of medications that relax the esophageal sphincter, thereby promoting reflux, may increase risk of adenocarcinomas of the esophagus and gastric cardia *(88)*. Histamine H_2 receptor antagonists used routinely for treatment of peptic ulcer and gastroesophageal reflux disease have also been proposed as an etiological factor *(89)*. In a 1995 report, Chow et al. *(90)* examined the relationship between reflux disease and its treatment to risk of adenocarcinomas of the esophagus and gastric cardia. Significant increased risks of adenocarcinoma were associated with esophageal reflux (OR = 2.1, 95% CI 1.2–3.6), hiatal hernia (OR = 3.8, 95% CI 1.9–7.6), and esophagitis/esophageal ulcer (5.0, 95% CI 1.5–16.4). Although a fourfold increased risk was associated with four or more prescriptions for H_2 antagonists, the odds ratio was reduced to 1.5 (95% CI 0.4–5.4) after adjusting for predisposing conditions. The relationship with use of anticholinergics adjusted for number of conditions was actually inverse: risk decreased with increasing number of prescriptions (p-trend = 0.08). The study findings support the elevated risk of adenocarcinoma conferred by reflux disease but indicate that the mechanism is not strongly related to treatment of reflux. An interesting finding indicates an increased risk of esophageal adenocarcinoma among long-term users of drugs that containing theophylline *(91)*. The significance of this finding is linked to the rising incidence of asthma and increasing use of asthma medications in the general population and its association with reflux disease. However, a Swedish cohort study followed 92,986 adult patients who were hospitalized for asthma from 1965 to 1994 for an average of 8.5 yr to evaluate their risk of esophageal adenocarcinoma *(92)*. The study findings suggested that the excess risks for the cancer were largely restricted to asthmatic patients who also had a discharge record of gastroesophageal reflux (standardized incidence ratio [SIR] = 7.5, 95% CI 1.6–22.0). No association was found for nonreflux asthmatic patients.

2.2.2. Tobacco and Alcohol

Two population-based studies of cancers of the esophagus and gastric cardia conducted in western Washington from 1983 to 1990 were analyzed to evaluate risk factors for adenocarcinoma compared to squamous cell carcinoma *(93)*. Use of alcohol and cigarettes were significantly associated with increased risk of both histological types, but the odds ratios were markedly higher for squamous cell carcinoma. For current smokers of 80 or more packs per year compared to nonsmokers, the odds ratios were 16.9 (95% CI 4.1–6.91) for squamous cell carcinoma and 3.4 (95% CI 1.4–8.0) for adenocarcinoma. Similarly, for persons who reported drinking 21 or more drinks per week compared to less than 7 drinks per week, the respective odds ratios were 9.5 (95% CI 4.1–22.3) and 1.8 (95% CI 1.1–3.1). Population-attributable risk estimates found that cigarette smoking and alcohol together accounted for 87% of the squamous cell carcinomas, whereas for adenocarcinoma, the estimates for cigarettes and alcohol consumption of seven or more drinks per week were 34 and 10%, respectively.

Estimates by Kabat et al. *(94)* of esophageal adenocarcinoma risk for alcohol and tobacco use were similar; for current smokers, the OR was 2.3 (95% CI 1.4–3.9), and for

alcohol drinkers who comsumed 4+ oz of whiskey-equivalents per week, the OR was 1.9 (95% CI 1.3–4.3). Brown et al. *(95)* also reported that tobacco and alcohol are likely etiological factors but conferred lower magnitude risk than that associated with squamous cell cancers. The odds ratios at the highest level of smoking (>40 cigarettes/d) and drinking (>29 drinks/wk) were 2.6 (p-trend < 0.01) and 2.8 (p-trend < 0.05), respectively. Their study included Caucasian men from Atlanta, Detroit, and New Jersey. Significantly increased risks were also found associated with history of ulcer, especially duodenal, and with low social class. The authors noted that although alcohol and tobacco use are associated with esophageal adenocarcinoma, they do not explain the increased incidence in these tumors in the United States.

In 1997, a multicenter study of esophageal and gastric cancers reported an increased risk of squamous cell carcinoma and adenocarcinoma of the esophagus and adenocarcinomas of all sites in the stomach among smokers *(96)*. Current smokers had a two- to threefold increased risk of adenocarcinomas of the esophagus and gastric cardia compared to a fivefold increased risk of squamous cell carcinoma of the esophagus. Although risk of these squamous cell tumors declined with duration of smoking cessation, risks of esophageal and cardia adenocarcinomas remained significantly elevated for more than 30 yr after cessation. This long lag suggests that the effect of tobacco on these tumors may be on tumor initiation.

2.2.3. Obesity and Diet

Two recent reports linked obesity to adenocarcinoma of the esophagus *(93,97)*. A threefold increased risk ($p < 0.01$) was observed at the highest level of body mass index (BMI) (>26.6 kg/m^2) compared to the lowest level of BMI in Caucasian men *(97)*. No significant associations were found for dietary fat, total calories, meals eaten per day, or consumption of coffee and tea. A protective effect was observed for high intake of raw fruit (OR = 0.4; $p < 0.05$) and vegetables (OR = 0.4; $p < 0.05$). Vaughan et al. *(93)* reported divergent associations for squamous cell carcinoma and adenocarcinoma with BMI. A significantly increased risk of adenocarcinoma was found at the highest decile of BMI (OR = 1.9, 95% CI 1.1–3.2), whereas body mass was inversely associated with squamous cell carcinoma. The population-attributable risk for BMI above the 50th percentile was 18% for adenocarcinoma. These observations are consistent with esophageal reflux associated with obesity. These reports were confirmed in a large multicenter, population-based, case–control study *(98)* in which obesity measured by BMI was found to be a strong risk factor for esophageal adenocarcinoma and a moderate risk factor for adenocarcinoma of the gastric cardia. The authors suggested that the increasing rates of adenocarcinomas of the esophagus and cardia may partly be explained by the increasing prevalence of obesity in the US population.

2.2.4. *Helicobacter pylori*

Peptic ulcer disease and gastric cancer of the antrum and body have been declining in recent decades *(1,99)*. In contrast, gastroesophageal reflux disease, Barrett's esophagus, and esophageal adenocarcinoma rates have increased rapidly, particularly in the Western countries. Recent studies *(100–102)* have suggested that this phenomenon may be related to the simultaneous fall in the prevalence of *H. pylori*. A Swedish cohort of 32,906 middle-aged subjects enrolled between 1974 and 1992 were followed through April 1999 *(101)*. *H. pylori* infection was determined through analysis of *H. pylori* antigen

in blood samples. A nested case–control study of 49 esophageal cancer cases and 10 matched sets of controls were included in the analysis. Unexpectedly, *H. pylori* seropositivity was present in 22.7% cases and 45.0% of controls. After adjusting for confounding factors, esophageal cancer was associated with *H. pylori* infection (OR = 0.29, 95% CI 0.12–0.67). The inverse association between *H. pylori* infection and cancer of the esophagus is stronger for esophageal adenocarcinoma (OR = 0.16) than for squamous cell carcinoma (OR = 0.41). However, the histologically specific associations did not reach statistical significance because of the small number of cases. This finding as well as several others have sometimes been reported as *H. pylori* infection "protects" against esophagus cancer *(103,104)*. It is possible that decreased gastroesophageal reflux among people infected with *H. pylori* reduces the risk for developing an esophagus cancer. However, factors responsible for the development of esophageal cancer may also create a hostile mucosal environment and cause the disappearance of *H. pylori* infection. Additional information is needed to clarify the nature of the relationship between *H. pylori* infection and esophageal cancer risk. The American and European guidelines for appropriate testing and treatment of *H. pylori* are provided at the end of the chapter. Currently, treatment is not recommended for patients presenting with gastroesophageal reflux disease.

3. CANCER OF THE STOMACH

A steady decline in gastric cancer has been apparent in many countries for the past several decades. The declining rates were first noted in the United States as early as 1930 *(105)* and have persisted into this century *(1)*. Survival rates have not appreciably changed *(1,106)*; therefore, the decline in deaths cannot be attributed to better treatment and prolonged survival but, rather, to actual declines in incidence that are now well-documented *(107)*. This decline, which is believed to reflect changes in environmental factors, has been referred to as an "unplanned triumph," because the shifts did not result from active medical or public health intervention and are believed to have resulted from large shifts in food processing and consumption *(108)* as well as a declining prevalence of *H. pylori* infection. It should be noted that the increase in esophageal adenocarcinoma that was documented in the previous section does include an increase in adenocarcinomas of the gastroesophageal junction and gastric cardia.

3.1. Histological Types

Adenocarcinomas account for more than 97% of gastric cancers, and studies of etiology are generally limited to this histological type *(109)*. Building on an earlier observation that gastric carcinomas were often accompanied by features found in intestinal epithelium *(110)*, Lauren *(111)* proposed a classification of adenocarcinomas into two subtypes: "intestinal" and "diffuse." Many, but not all, tumors can be classified into one of these subtypes, because some tumors contain characteristics of both types and others contain characteristics of neither. Diffuse carcinomas, sometimes referred to as "endemic," tend to occur with similar frequency worldwide, whereas the distribution of intestinal, or "epidemic," type of carcinomas tends to parallel the distribution of overall gastric cancer rates (i.e., this type is relatively more common in areas with high rates and lower where gastric cancer rates are low) *(112)*.

3.2. Risk Factors

3.2.1. *H. pylori*

Spiral-shaped bacteria in contact with gastric mucosa were first reported by Pel about 100 yr ago *(113)* and were ignored for the following 90 yr. In 1984, Marshall and Warren *(114)* reported isolating these bacteria in cultures of biopsies taken from patients with gastritis and peptic ulcers who were undergoing endoscopy. By 1994, the International Agency for Research on Cancer, World Health Organization, had determined that infection with *H. pylori* is carcinogenic to humans, and declared it a Group I carcinogen based on the large body of research developed during the 11-yr period *(115)*.

H. pylori infection is one of the most prevalent infections worldwide, with a range of 20 to 40% in developed countries and a range as high as 70 to 90% in some developing countries *(116,117)*. Prevalence increases with age, and no difference in seroprevalence has been found between males and females *(118)*. Socioeconomic status (including poor housing conditions, large family size, and low education attainment) are predictors of prevalence of infection as well as of gastric cancer.

Person-to-person spread is believed to be key in acquisition of infection, which generally occurs in childhood. Intrafamilial spread is supported by studies demonstrating a high prevalence of *H. pylori* among family members of an infected individual. A recent study from Greece examined the entire genome of *H. pylori* strains from 32 members of 11 families. The homology of the *H. pylori* genome in members of the same family was striking and strongly supported the hypothesis of transmission from person to person or from a common source *(119)*.

The role of *H. pylori* in gastric carcinogenesis has been explored in correlational and case–control studies; however, this approach has yielded equivocal results, largely because of difficulties in determining temporality *(115)*. Three different cohort studies provided material for nested case–control analyses that resolved the issue of temporality. *H. pylori* infection was determined by IgG antibodies in serum that was collected at the time of cohort enrollment, which occurred 6 to 14 yr earlier. Forman et al. *(120)* found an approximate threefold increased risk of subsequent gastric cancer in a cohort of Welsh men; Parsonnet et al. *(121)* reported a relative risk of 3.6 (95% CI 1.8–7.3) in a cohort of men and women in California; and Nomura et al. *(122)* found a sixfold significantly increased risk in Japanese-American men living in Hawaii.

The mechanisms by which *H. pylori* infection increases gastric cancer risk are the focus of ongoing investigation. *H. pylori* infection, the main cause of chronic gastritis, has been demonstrated to decrease the concentration of ascorbic acid in gastric juice *(123–126)*. *H. pylori* infection also is associated with varying degrees of inflammation *(127)*. In inflammatory states, nitric oxide may be generated and interact with reactive oxygen species to form new cytotoxic compounds *(128,129)*. Therefore, *H. pylori* infection has the potential to increase oxidative stress and decrease antioxidant capacity.

The vast majority of individuals who are infected with *H. pylori* (80%) manifest no clinical symptoms, and fewer than 5% develop gastric cancer *(130)*. Therefore, an understanding of the determinants of cancer development among infected persons is important. Recent research suggests that the interplay of bacterial virulence factors and host susceptibility factors play a key role in the subsequent development of gastric cancer *(131)*. Cytotoxin-associated antigen (CagA) and vacuolating toxin (VacA) are associated with cancer outcomes. Risks of gastric adenocarcinoma and gastric atrophy

(a premalignant condition) have been associated with $CagA^+$ and $VacA^+$ strains compared to negative strains *(132–134)*. $BabA2^-$positive strains are also associated with increased gastric cancer risk. BabA is an adherence factor that causes the bacteria to bind to Lewisb blood group antigens on gastric epithelial cells. Host genetic factors that influence the immune response to infection are also believed to play a role in this process. Current research suggests that a pro-inflammatory response to *H. pylori* is associated with an increased risk of noncardia gastric cancer. Pro-inflammatory genotypes of tumor necrosis factor-α and interleukin-10 have been associated with a doubling of gastric cancer risk *(135)*.

3.2.2. Tobacco and Alcohol

Although both tobacco and alcohol use are weakly associated with an increased risk of gastric cancer, the strength and magnitude of the association is much less clear than that for esophageal cancer.

Early case–control studies of gastric cancer and alcohol intake were equivocal, with some reporting positive associations *(136,137)* and others reporting none *(138,139)*. Continued study has yielded similar mixed results. Correa et al. *(140)* found twofold elevations in the risk of gastric cancer at the highest level of alcohol intake for both Caucasian and African-Americans in Louisiana. After controlling for other risk factors, wine (OR = 2.10, 95% CI 1.13–3.89) and hard liquor (OR = 1.95, 95% CI 1.14–3.34) were significantly associated with risk in Caucasians but not in African-Americans. In 1990, a report regarding stomach cancer in Los Angeles males also found an increased risk (OR = 3.0, 95% CI 1.1–8.7) at the highest level of total ethanol intake as well as significant risks for daily consumption of beer *(141)*. The effect of alcohol was stronger for cancer of the gastric cardia than at other sites.

A twofold increased risk of stomach cancer was found for beer consumption in a German study, whereas wine and hard liquor were associated with decreased risk *(142)*. This is in contrast to a French study that reported a very large relative risk (RR = 6.9, 95% CI 3.3–14.3) associated with heavy use of red wine *(143)*.

However, two cohort studies have suggested that alcohol is not an independent risk factor for gastric cancer. Nomura et al. *(144)* found no increased risk of gastric cancer associated with consumption of beer, wine, or hard liquor in Japanese-American men living in Hawaii. Similarly, Kneller et al. *(145)* found no association for total alcohol or for any specific cancer type.

More consistent findings have linked smoking to a 1.5- to 3-fold increased risk of gastric cancer *(140–145)*; however, the overall increased risk has sometimes failed to demonstrate a dose–response *(136,144,146)*. The cohort study by Kneller et al. did find significant increases in risk with both increasing the number of cigarettes smoked per day and pack-years of smoking *(145)*. At the highest number of pack-years, the relative risk was 2.3 (95% CI 1.23–4.33), and for current use of 30 or more cigarettes per day, the relative risk was 5.8 compared to nonsmokers. Although age at death did not significantly modify risk, the association with smoking was stronger for younger cases. The authors suggested that this finding may reflect a higher proportion of adenocarcinomas of the gastric cardia at younger ages and a stronger relationship between smoking and cancers of the cardia than with cancers of other sites in the stomach.

The evidence on tobacco smoke and cancer risk that accumulated between 1986 and 2002 was recently reviewed by a work group assembled by the International Agency for

Research on Cancer *(147)*. For stomach cancer, the conclusions were that current smokers were at increased risk of both cardia and noncardia stomach cancer and that the association between tobacco smoking and gastric cancer risk is independent of alcohol consumption and *H. pylori* infection. However, the absolute risk tends to be higher among smokers who are *H. pylori*-positive *(148)*. The proportions of gastric cancer attributable to tobacco smoking (a modifiable risk factor) are about 17 and 11% in men and women, respectively, in developed countries and 11 and 4% for men and women, respectively, in developing countries.

3.2.3. Salted, Pickled, and Smoked Foods

In animal studies, salt has been demonstrated to enhance gastric carcinogenesis *(149–152)*. It has been suggested that the action of salt as a gastric mucosal irritant facilitates the action of carcinogens, and, therefore, salt acts as a cocarcinogen *(153)*.

Epidemiological studies have also suggested an increased risk of gastric cancer associated with high salt intake when salted and pickled foods are included in total intake. Death rates throughout regions of Japan *(154)* were found to be correlated with consumption of salted fish and vegetables. A geographical correlation was also demonstrated in China *(155)*. Consumption of salt-cured meats, salted fish, and other salt-preserved foods has been associated with increased risk in case–control studies worldwide *(156–159)*. Several studies have also reported associations with the addition of salt to foods *(156,160)* or a reported "heavy intake" *(140,161)*.

Many of the strongest findings have been noted in areas of the world where there is a wide range of intake, including very high levels, such as in Korea *(158)*. In a recent nested case–control analysis in a California study population, Friedman and Parsonnet *(162)* failed to find evidence that routine salting led to increased risk. "Heavy" salt intake in US populations may be quantitatively less than "heavy" intake in other areas of the world and may not be sufficient to demonstrate an increased risk. For example, salted fish and vegetables in Japan may contain up to 30% sodium chloride (NaCl) compared to isotonic saline, which contains 0.8% *(153,154,162)*. One of the difficulties in assessing the role of salt is determination of "exposure," for which many assessments have been subjective. A step forward in research was the use of a salt taste test *(163)*. Preference for salty taste was objectively assessed using diluted rice cereal (gruel) that contained 0.1, 0.3, 0.5, 0.7, and 0.9% NaCl. The five different diluted rice cereals were randomly arranged, and the subjects were asked to select a preferred concentration. People with gastric cancer were significantly more likely ($p < 0.01$) to prefer the cereals with high concentrations of salt (>0.5%). In this study, persons who were infected with *H. pylori* and who had a high-salt preference had a 10-fold higher risk of early gastric cancer than uninfected persons who preferred low salt concentrations ($p < 0.05$).

Numerous *N*-nitroso compounds have demonstrated carcinogenicity *(164)*. Based on studies of premalignant lesions of the stomach, it has been hypothesized that intragastric synthesis of *N*-nitroso compounds is a factor in the gastric carcinogenic process *(165)*.

Two recently reported studies evaluated factors associated with in vivo nitrosamine formation in humans using the test developed by Ohshima and Bartsch *(166)*, which measures urinary excretion of noncarcinogenic *N*-nitrosoproline after ingesting a given dose of proline. Mirvish et al. *(167)* found that men in rural Nebraska who drank water from private wells with a high-nitrate content excreted significantly higher *N*-nitrosoproline

than men who drank water with a low-nitrate content. Their findings paralleled those of a study in Denmark *(168)*. Sierra et al. *(169)* used the nitrosoproline test in children in Costa Rica who lived in high- and low-risk areas for stomach cancer. They found that the concentration excreted by children in the high-risk area was significantly greater ($p < 0.04$) compared to children from the low-risk area. They also found that excretion was markedly reduced when ascorbic acid (an inhibitor of nitrosation reactions) was administered with the proline.

Associations between gastric cancer and dietary intake of nitrate, nitrite, and preformed nitroso compounds are suggestive *(170–175)*, but the validity of such indices is not well-established given the multiple sources—including food, water, and endogenous formation.

3.2.4. Fruits and Vegetables

Table 2 presents an extensive compendium of dietary studies of gastric cancer *(138,140–142,144,145,158,161,170–191)*. The strong, consistent inverse association between consumption of fruits and vegetables is abundantly clear. Of the 26 studies described that specifically examined foods and food groups, 24 found a decreased risk of stomach cancer associated with high intake of one or more fruits and vegetables, and the vast majority were statistically significant, with up to twofold reductions in risk. Only two studies reported an increased risk of gastric cancer associated with fruits *(145)* or vegetables *(157)*, and their findings cast little doubt on the apparent protective effect of fruits and vegetables. The findings of Tajima and Tominaga *(157)* stand in contrast to many case–control studies in Japan and elsewhere, and the study by Kneller et al. *(145)* was based on a very limited dietary questionnaire, which increased the likelihood of misclassification.

Norat and Riboli conducted a meta-analysis of published case–control and cohort studies of fruit and vegetable consumption *(192)*. The pooled analysis of all studies found that high intake of vegetables was associated with significantly decreased risk of stomach cancer. Fruit intake was associated with a significantly reduced risk of gastric and esophageal cancers. Estimates of the proportion of stomach cancers worldwide that are potentially preventable by increasing fruit and vegetable consumption are up to 50%.

3.2.5. Micronutrients

Fruits and vegetables serve as dietary sources of a plethora of vitamins, minerals, fiber, and the less well-studied trace compounds. Many of these are highly correlated with one another, particularly when exposure is based on dietary assessment; therefore, a finding attributed to one may actually reflect the effect of another constituent from the same foods. Thus, the strongest findings are based on biochemical studies (e.g., blood levels prior to cancer onset) and chemoprevention trials, which actually test the efficacy of specific micronutrients in prevention. Vitamin C, β-carotene, and vitamin E/selenium are the micronutrients that are most strongly believed to be associated with reduced risk of gastric cancer based on currently published studies.

Findings from dietary estimates of intake are also included in Table 2. Relatively high consumption of vitamin C and β-carotene is consistently associated with reduced risk of gastric cancer *(140,142,161,170,172–174,176,179,183,185,186)*. Serological assessment also supports a role. Prospective studies that have evaluated vitamin C are

Table 2
Selected Epidemiological Studies of Diet and Stomach Cancer Risk

Study (reference)	*Population*	*Number of cases/control or cohort size*	*Food or nutrient*	*Relative risk (high vs low intake)*
Case–Control				
Meinsma *(176)*	Holland	340/1060	Vitamin C	Inverse association
			Citrus fruit	$p = 0.1$ males $p = 0.001$ females
Higginson *(177)*	United States	93/279	Dairy foods	0.6
			Fresh fruits	Inverse association
			Raw vegetables	Inverse association
Haenszel et al. (*178*)	Japanese in Hawaii	220/440	Tomatoes	0.4 ($p < 0.05$)
			Celery	0.4 ($p < 0.05$)
			Corn	0.5 ($p< 0.05$)
			Onion	0.5 ($p < 0.05$)
			Lettuce	0.8 (NS)
			Western vegetables combined	0.4 ($p < 0.05$)
Graham et al. *(138)*	United States	276/2200	Lettuce	0.64 (p-trend < 0.01)
Bjelke *(179)*	Norway and United States	162/1394	Vegetable index (Norway)	Inverse association (Norway and United States)
		259/1657	Vitamin C	Inverse association (Norway and United States)
			Fruits & vegetables (United States)	Inverse association (Norway and United States)
Haenszel et al. *(180)*	Japan	783/1566	Fruit	0.7 ($p < 0.05$)
			Plum and pineapple	0.7 ($p < 0.01$)
			Celery	0.6 ($p< 0.01$)
			Lettuce	0.7 ($p< 0.01$)

(continued)

Table 2 *(continued)*

Study (reference)	*Population*	*Number of cases/control or cohort size*	*Food or nutrient*	*Relative risk (high vs low intake)*
Correa et al. *(140)*	United States	391/391	Vitamin C	0.50 (p–trend < 0.05) Caucasians
				0.33 (p–trend < 0.001) African-Americans
			Fruit index	0.47 (p–trend < 0.005) Caucasians
				0.33 (p-trend < 0.001) African-Americans
			Vegetable index	0.50 (p-trend < 0.05) African-Americans
			Smoked foods	1.98 (p–trend < 0.025) African-Americans
Risch et al. *(170)*	Canada	246/246	Vitamin C	0.43 (p–trend = 0.099)
			Citrus fruit	0.75 (p–trend = 0.006)
			Nitrite	2.61 (1.61–4.22)
			Carbohydrates	1.53 (1.07–2.18)
Trichopoulos et al. *(181)*	Greece	110/100	Lemons	0.24 (p-trend < 0.01)
			Oranges	0.33 (p–trend < 0.01)
			Pasta	3.42 (p–trend < 0.001)
			Brown bread	0.79 (p–trend < 0.01)
			Onions	0.68 (p–trend < 0.001)
Tajima and Tominaja *(157)*	Japan	93/186	Oranges	0.9 (NS)
			Other fruit	1.4 (NS)
			Spinach	2.5 (p < 0.05)
			Cabbage	2.2 (p < 0.01)
			Green pepper	2.0 (p < 0.01)
Jedrychowski et al. *(182)*	Poland	110/110	Fruit	0.3 (0.1–0.6)
			Vegetables	0.6 (0.3–1.4)

LaVecchia et al. *(161)*	Italy	206/474	Vitamin C	0.46 ($p < 0.001$)
			Fruits, index	0.53 (p–trend < 0.01)
			Citrus fruit	0.58 (p–trend < 0.01)
			Green vegetable index	0.33 (p–trend < 0.001)
			Ham	1.6 ($p = 0.04$)
			Polenta	2.32 ($p = 0.007$)
			β–carotene	0.39 ($p < 0.001$)
You et al. *(183)*	China	564/1131	Vitamin C	0.5 (0.3–0.6)
			Fresh fruit	0.4 (0.3–0.6)
			Fresh vegetables	0.6 (0.4–0.8)
Buiatti et al. *(184)*	Italy	1016/1159	Raw vegetables	0.6 (p–trend < 0.001)
			Citrus fruits	0.6 (p–trend < 0.001)
			Other fresh fruits	0.4 (p–trend < 0.001)
Graham et al. *(185)*	United States	293/293	Raw vegetables	0.43 (0.23–0.78)
			Fruits	No association
			Vitamin C	No association
			Carotene	0.79 (0.63–0.98)
			Sodium	1.51 (1.20–.91)
			Retinol	1.47 (1.17–1.85)
			Fat	1.37 (1.08–1.74)
Chyou et al. *(171)*	Japanese in Hawaii	111/361	Vegetable index	0.7 (p–trend < 0.001)
			Fruit index	0.8 (p–trend = 0.20)
			Nitrite	No association
Buiatti et al. *(172)*	Italy	1016/1159	Vitamin C	0.5 (p–trend < 0.001)
			α–tocopherol	0.6 (p–trend < 0.01)
			β–carotene	0.6 (p–trend < 0.01)
			Protein	2.6 (p–trend < 0.001)
			Nitrites	1.9 (p–trend 0.001)
Wu–Williams et al. *(141)*	United States	137/137	Fruit index	0.7 (NS)
			Beef	1.6 (1.0–2.6)

(continued)

Table 2 *(continued)*

Study (reference)	*Population*	*Number of cases/control or cohort size*	*Food or nutrient*	*Relative risk (high vs low intake)*
Buiatti et al. *(173)*	Italy	923/1159	Vitamin C	0.5 (0.3–0.6) intestinal-type 0.5 (0.3–0.7) diffuse-type
			Citrus fruits	0.5 (0.4–0.7) intestinal-type 0.6 (0.4–0.9) diffuse type
			Other fresh fruits	0.5 (0.4–0.7) intestinal-type 0.4 (0.3–0.6) diffuse-type
			Raw vegetables	0.6 (0.4–0.8) intestinal-type 0.6 (0.4–0.9) diffuse-type
			α–tocopherol	0.5 (0.3–0.8) intestinal-type 0.5 (0.2–0.8) diffuse-type
			β–carotene	0.7 (0.5–0.8) intestinal-type 0.6 (0.4–0.7) diffuse-type
			Nitrites	1.8 (1.2–2.8) intestinal-type 2.8 (1.5–5.0) diffuse-type
			Protein	2.4 (1.02–5.6) intestinal-type 5.8 (1.8–1.84) diffuse type
Negri et al. *(186)*	Italy	564/6147	Green Vegetables	0.4 (0.3–0.6) (p–trend < 0.001)
			Fruit	0.4 (0.3–0.5) (p–trend < 0.001)
Boeing et al. *(142)*	Germany	143/579	Vitamin C	0.37 (p–trend < 0.01)
			Cheese	0.44 (p–trend < 0.01)
			Processed meat	1.74 (p–trend < 0.01)
			Whole wheat bread	0.37 (p–trend < 0.001)
Gonzalez et al. *(187)*	Spain	354/354	Cooked vegetables	0.5 (trend p = 0.02)
			Non–citrus fresh fruits	0.6 (p-trend = 0.006)
			Dried fruits	0.4 (0.2–0.8)
			Meat	0.6 (p–trend = 0.02)
Hoshiyama & Sasaba *(188)*	Japan	251/483	Fruits	Inverse association
			Raw vegetables	Inverse association
			Pickled vegetables	Increased risk

LaVecchia et al. *(189)*	Italy	723/2024	β–carotene	0.38 (p-trend< 0.001)
			Vitamin C	0.53 (p-trend < 0.001)
			Methionine	2.40 (p-trend < 0.001)
Lee et al. *(158)*	Korea	213/213		
Hansson et al. *(190)*	Sweden		Total Vegetables	0.58 (0.37–0.89) (p-trend = 0.01)
			Citrus fruits	0.49 (0.29–0.81) (p-trend = 0.004)
Hansson et al. *(174)*	Sweden		Vitamin C	0.47 (0.30–0.76) (p-trend = 0.003)
			β–carotene	0.73 (0.45–1.18) (p-trend = 0.10)
			Nitrates	0.97 (0.60–1.59) (p-trend = 0.99)
Lopez–Carrillo et al. *(191)*	Mexico	220/752	Chili peppers (ever, never)	5.49 (2.72–11.06)
			Chili peppers (high vs none)	17.11 (7.78–37.59)
Gonzales et al. *(175)*	Spain	354/354	Nitrosomines	2.1 (p-trend = 0.007)
			Fiber	0.35 (p-trend < 0.001)
			Folate	0.50 (p-trend = 0.008)
			Vitamin C	0.58 (p-trend = 0.017)
Cohort				
Nomura et al. *(144)*	Japanese in Hawaii	150/7990	Fruit index	0.8 (0.5–1.3)
			Fried vegetables	0.8 (0.4–1.6)
Kneller et al. *(145)*	United States	75/17,633	Fruit index	1.5 (p-trend, NS)
			Vegetable index	0.9 (p-trend , NS)
			Carbohydrates	1.6 (p-trend < 0.05)

scant because vitamin C deteriorates quickly unless specimens are acid-stabilized prior to freezing *(193)*. The Basel study*(194)*, a large well-conducted cohort study, did have such material available. Mean plasma vitamin C was significantly lower in persons who died of cancer than in survivors (47.61 ± 1.78 mol/L vs 52.76 ± 0.44 mol/L, respectively; $p < 0.01$). The findings were also significant ($p < 0.05$) for persons who subsequently died of stomach cancer, and their blood levels were even lower (42.86 ± 4.88). Low plasma levels of vitamin C were associated with a relative risk of 2.38 for gastric cancer. Low plasma levels of β-carotene were similarly associated with significantly increased risk of overall mortality from cancer ($p < 0.01$) and cancer of the stomach ($p < 0.01$), which had a relative risk of 2.95. No association was observed between plasma levels of vitamin A or E and gastric cancer.

Haenszel et al. *(195)* measured serum micronutrient levels in persons with various premalignant gastric lesions. β-carotene levels in both men and women and vitamin E levels in men were significantly lower in subjects with gastric dysplasia than in subjects with normal mucosa or less advanced lesions.

A recent report from Japan *(196)* evaluated prediagnostic serum selenium and zinc levels and found no excess risk of stomach cancer in those with the lowest levels of selenium (OR = 1.0) or zinc (OR = 1.2).

To date, the most compelling evidence for specific micronutrients in chemoprevention of gastric cancer comes from the previously described population trial in China *(73)*, which found a significant reduction in stomach cancer mortality among persons taking a combination of β-carotene, vitamin E, and selenium. No reduction in risk was observed among persons taking vitamin C; however, there was no attempt in this trial to eradicate *H. pylori*—which is known to decrease the concentration of ascorbic acid in gastric juice—either by increased oxidation, impaired secretion from blood into the gastric cavity, or both *(122,124–126)*. In 2000, another chemoprevention trial was conducted in a high-risk population in the Andes mountain area of Colombia. After 6 yr of follow-up study, participants who were treated with either ascorbic acid, β-carotene, or anti-*H. pylori* therapy, individually or in combination, were significantly more likely to have regression of gastric premalignant lesions *(163)*.

4. RECOMMENDATIONS

Primary prevention of esophageal and, to a lesser degree, stomach cancer obviously begins with prevention of tobacco use by teenagers and cessation among addicted adults. Physician prompting and participation in smoking cessation efforts have proved effective. Nicotine replacement, in conjunction with behavioral modification, may improve the success rate for smokers who are attempting to quit. More nicotine replacement methods are available now than ever before. These include gum, which was the first type of replacement method developed; a patch by which nicotine is absorbed through the skin; an inhaler; and a nasal spray. These methods can be used to deliver gradually reduced doses of nicotine without exposure to the myriad harmful chemicals contained in tobacco smoke.

Several non-nicotine-containing pharmacological approaches to cessation have been attempted; however, one has been demonstrated to have serious adverse side effects associated with use, and others, which are used in treatment of specific disorders, have not yet been approved by the Food and Drug Administration for use in cessation. These options are expected to develop with additional research.

Table 3
Websites of Organizational Nutritional Guidelines

American Cancer Society	http://www.cancer.org
National Cancer Institute	http://www.nci.nih.gov http://5aday.com
American Heart Association	http://www.americanheart.org
American Diabetes Association	http://www.diabetes.org

A reduction in tobacco use by teenagers has proved a persistent challenge, because educational programs are offset by well-funded, effective, targeted marketing and promotion by tobacco companies. The terms of the 1998 Tobacco Settlement have the potential to reduce the impact of industry marketing and promotions, particularly if complemented by the use of settlement funds for countermarketing campaigns in those few states that have chosen this approach. Limiting alcohol consumption to moderate levels is particularly important for smokers, and physicians should actively counsel patients accordingly.

Intake of fresh fruits and vegetables in the United States continues to fall short of the recommended "5-A-Day" *(197)*. More recent programs have encouraged even higher levels of consumption (e.g., "9-A-Day"). Increased consumption should continue to be promoted, and benefits are expected to accrue in reduced rates of both of these upper digestive tract cancers as well as other epithelial tumors. Effective population-based approaches are important, and because dietary patterns often are established in childhood, promotion of healthy choices in school-based food-service programs is an opportunity that should not be missed.

The current dietary recommendations of the American Cancer Society, the American Heart Association, and the National Cancer Institute are remarkably similar in direction but differ in specificity. The most consistent general recommendations for prevention of the major chronic diseases in this country, including these cancers, are to eat more fruits, vegetables, and grains; eat less high-fat foods and meats; and to be active and stay fit. Website addresses with up-to-date material regarding dietary recommendations are included for reference in Table 3.

The efficacy of vitamin/mineral supplements has not yet been completely established in clinical trials; however, in case–control and cohort studies, the individuals in the highest level of intake of specific micronutrients often combine high dietary intake with supplements. With the obvious caution to avoid excessive intake, a multivitamin/mineral supplement or specific antioxidant supplement may complement dietary intake, particularly among persons with excessive oxidative stress, such as smokers.

In 2000, the original European *H. pylori* treatment guidelines were updated in a second Maastricht Consensus Report *(198)*. The guidelines recommend a "test and treat" approach in adult patients under age 45 yr who present in primary care settings with persistent dyspepsia, after excluding those with predominantly gastroesophageal reflux disease symptoms, nonsteroidal anti-inflammatory drug users, and those with alarm symptoms requiring prompt endoscopic investigation (i.e., unexplained weight loss, dysphagia, recurrent vomiting, digestive bleeding or anaemia, abnormal physical examination, malabsorption, and concomitant disease with possible digestive involvement).

Additional recommendations from this report include:

- The urea breath test or stool antigen test are recommended as the diagnostic tests of choice.
- *H. pylori* eradication is recommended in all patients with peptic ulcers, low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma, or atrophic gastritis and in patients undergoing postgastric cancer resection.
- Eradication is also strongly recommended in infected patients with a first-degree family history of gastric cancer.

The American College of Gastroenterology issued guidelines for the management of *H. pylori* in 1998 *(199)*. Their recommendations include the following:

- Diagnostic testing for *H. pylori* infection should be performed only if treatment is intended.
- Testing for *H. pylori* infection is indicated in patients with active peptic ulcer disease, a history of documented peptic ulcer, or gastric MALT lymphoma.
- Testing for *H. pylori* infection is not indicated in asymptomatic individuals with no history of peptic ulcer disease or in patients on long-term treatment with a proton pump inhibitor (PPI) for gastroesophageal disease.
- There is no conclusive evidence that eradication of *H. pylori* will reverse the symptoms of nonulcer dyspepsia. Patients may be tested for *H. pylori* on a case-by-case basis, and treatment may be offered to those who test positive.
- The diagnostic test to use in a particular patient depends on the clinical setting, particularly if upper GI endoscopy is performed.
- When endoscopy is indicated, the test of choice is a urease test on an antral biopsy. If a biopsy urease test is negative, *H. pylori* infection may be diagnosed by histology or serology. Biopsy urease tests have reduced sensitivity in patients taking PPIs and in patients with recent or active bleeding. Histology generally is not considered necessary and is expensive. When endoscopy is not performed, an office-based serological test is the least expensive means of evaluating for evidence of *H. pylori* infection.
- The highest eradication rates are achieved with the following regimens:

    1. A PPI, clarithromycin, and either amoxicillin or metronidazole for 2 wk.
    2. Ranitidine; bismuth citrate; clarithromycin; and either amoxicillin, metronidazole, or tetracycline for 2 wk.
    3. A PPI, bismuth, metronidazole, and tetracycline for 1 to 2 wk.

- Currently, routine posttreatment testing is recommended only in patients with a history of ulcer complications, gastric MALT lymphoma, or early gastric cancer. Patients with recurrent symptoms after treatment of *H. pylori* infection also need further evaluation.

Although the spectrum of clinical outcomes associated with *H.pylori* infection is wide-ranging, from asymptomatic to gastric cancer, treatment and eradication of infection as recommended previously is important because the cofactors that predispose an infected individual to gastric cancer have not yet been established.

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3 Non-Nutritive Components in Foods as Modifiers of the Cancer Process

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KEY POINTS

- Dietary habits are instrumental in about 40% of cancers in men and 60% in women.
- Fruit and vegetable consumption is associated with decreased risk of several cancers.
- Fruits and vegetables contain many non-nutritive as well as nutritive compounds including carotenoids, dithiolthiones, flavonoids, glucosinolates, indoles, isothiocyanates, monoterpenes, phenols, sterols, sulfhydryls, and vitamins including folate, C, and E.
- Anticarcinogenic actions from dietary components in fruits and vegetables include the induction of detoxification enzymes, blockage of carcinogen formation (such as nitrosamines), shifts in hormone homeostasis, slowing of cell division, induction of apoptosis, depression in angiogenesis, and several others.
- There are critical interrelationships between diet, environment, and genetics that affect cancer risk.
- In addition to fruits and vegetables, teas, spices, and herbs consumed in the diet have been associated with reducing cancer risk in cell culture and animal models; epidemiological studies show similar associations, but there are very few intervention studies to date.

1. INTRODUCTION

Cancer, once thought to be an inevitable consequence of the aging process, is now considered to be primarily determined by the interaction of environmental factors, including dietary habits, with genetic, epigenetic, and posttranslational events involved in the cancer process *(1–6)*. Because dominantly inherited or familial cancers probably contribute only a small percent of total cases, it is of paramount importance to identify those environmental modulators that influence nonfamilial risks *(1,2,7)*. Dietary habits are possibly a variable that markedly influences nonfamilial cancer risk. Some have estimated that dietary habits are instrumental in about 60% of cancers in women and about 40% of cancers in men *(8)*. Although these are significant contributions, the true

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effect depends on the individual's genetic profile, the particular neoplasms, and the composition of the entire diet.

Although variability exists, fruit and vegetable consumption has often been inversely linked with the incidence of cancer *(9–17)*. The reason for variability remains obscure but may relate to oxidative balance *(13,16)* or other physiological changes as indicated in this chapter. Variations in pro- and antioxidant conditions that might arise from the absence or presence of food components is recognized as an influence on several essential cellular functions, including gene expression profiles *(18,19)*. This homeostasis is unquestionably complex, as evident by the sensitivity of several kinases and transcription factors to rather subtle shifts in redox status *(20,21)*.

Almost a decade ago, Serdula et al. *(22)* reported that men and women living in various regions of the United States consumed an average of 3.3 and 3.7 servings of fruit and vegetables per day, respectively. More recently, based on a telephone survey, mean daily servings of fruits and vegetables consumed by rural African-Americans were found to be 3.7 *(23)*. In addition, in 1999, Thompson et al. *(24)* reported that daily consumption by 15,060 adults averaged 3.6 servings based on a seven-item food frequency questionnaire. Collectively, although there is a fair amount of agreement about average intakes, the actual number of servings depends on several societal factors, including cost and availability, as well as several personal determinants such as age, education, and race. Unquestionably, variation in fruit and vegetable consumption is recognized to influence an individual's redox status *(17,20,25)*. Unfortunately, few Americans (10–20%) consume the recommended five or more daily servings of fruits and vegetables *(22)*. Thompson et al. *(17)* suggested that even these individuals may not be able to meet all oxidative challenges. Their studies revealed that women whose daily vegetable and fruit consumption increased from 5.8 to 12 servings exhibited a marked reduction in 8-hydroxydeoxyguanosine in DNA isolate from peripheral lymphocytes, a biomarker of oxidative cellular damage. Overall, the linkages between fruit and vegetable consumption and reduced cancer risk serve as sufficient evidence for the continued examination of individual foods or dietary components as modulators of the initiation, promotion, or progression stages of carcinogenesis.

A large number of agents with antioxidant properties are found in fruits and vegetables, including carotenoids, dithiolthiones, flavonoids, glucosinolates, indoles, isothiocyanates, monoterpenes, phenols, sterols, sulfhydryls, and vitamins (including folate, C, and E). These dietary components likely have both complementary and overlapping mechanisms of action, including the induction of detoxification enzymes, blockage of carcinogen formation (such as nitrosamines), shifts in hormone homeostasis, slowing of cell division, induction of apoptosis, depression in angiogenesis, and several others.

Although several macronutrients likely are involved in the cancer process, they do not appear to totally explain the worldwide variance in cancer risk. Furthermore, it is possible that their impact is markedly influenced by several physiologically important dietary constituents. A host of nonessential constituents continue to emerge as key modulators of cancer risk. Collectively, these findings have highlighted the new and intriguing term *functional foods*, a name based on the ability of selected foods to have health benefits over and beyond the basic nutrition provided. These so-called functional foods continue to captivate the interest of scientists and legislators and, most importantly throughout the world, the consumer *(26–28)*. Although the term functional foods

Table 1
Potential Anticarcinogenic Compounds in Fruits, Vegetables, and Spices

Compound	*Food source*
Cinnamic acid	Fruit, vegetables, coffee beans
Flavonoids	Vegetables, fruits, citrus
Flavones	Fruit, celery, parsley
Flavonols	Vegetables, grains, onions, tea
Catechins	Tea
Flavanones	Citrus, grapefruit
Isoflavones	Soybean
Anthocyanidins	Grapes, cherry, raspberry
Indoles	Cruciferous vegetables
Isothiocyanates	Cruciferous vegetables
Lignans	Grains, flax
Organosulfur	*Allium* vegetables: garlic and onions
Terpenes	Citrus, spices

has no legal meaning, it signifies a proactive movement that deals with the health benefits of foods. Certainly, this is a refreshing change to the largely negative campaigns that have been the norm for providing diet and health information to the general public during the past few decades.

Clearly, variability in detecting an association between dietary practices and cancer risk is logical because such a relationship must depend on a host of environmental and genomic factors. The complexity of this issue becomes evident when one considers the multitude of interactions that can occur among the many food components. Likewise, because all metabolical and phenotypical characteristics are linked by heredity, it is almost inconceivable that a simple solution will alter susceptibility equally in all individuals. A greater understanding of the interrelationships among diet, environment, and genomics is needed to determine who might benefit most from changes in eating behavior. Acquiring this information will be key in producing tailored dietary recommendations that can assist in minimizing cancer risk in target populations *(29)*.

To date, more than 500 compounds have been suggested as potential modifiers of cancer. Some of the major antioxidant constituents of fruit, vegetables, and beverages are derived from phenolic phytochemicals synthesized through the shikimate pathway from tyrosine and phenylalanine *(30,31)*. Many of these exist as *O*-glycosides and *O*-methyl conjugates. Some are broadly presented in Table 1.

Cinnamic acid, widely found in fruits and vegetables, is a transformation product of phenylalanine produced by the action of phenylalanine-ammonia lyase. Isoflavonoids, flavonoids, and lignans are additional plant constituents that make up the three principal classes of phytoestrogens consumed by humans. Soy, a staple for many Asians, is a major source of the isoflavonoids daidzein and genistein,.

Flavonoids are also abundantly present in fruits. Quercetin and kaempferol are two commonly found flavonoids that are particularly profuse in apples, onions, and tea leaves. Plant lignans are present in many cereal grains, fruits, and vegetables and give rise to the mammalian lignans enterodiol and enterolactone. The richest sources of lignan precursors, such as secoisolariciresinol and matairesinol, are linseed (flaxseed)

and other oil seeds. *Allium* foods—including garlic, onions, and leeks—provide a host of organosulfur compounds that may influence health.

Terpenes are a group of hydrocarbons made up of building blocks of isoprene (C_5H_8) units that are widespread in nature. Most occur in plants as constituents of essential oils. Monoterpenes are composed of two units such as limonene, citral, and camphor, whereas sesquiterpenes are made up of three units and include compounds such as humulene, which is a hops aromatic. Carotene is an example of an 8-isoprene or tetraterpene unit.

Fruit and vegetable consumption is not the only dietary factor that can influence cancer risk. Ingestion of green and black tea, herbs, and spices has been reported to be inversely associated with cancer risk *(25,32–35)*. Some of these food items and their associated non-nutritive components are also addressed in subsequent parts of this chapter.

This chapter is limited to a few nonessential dietary components or for those for which substantial documentation exists about an effect on the cancer process and for those in which a plausible mechanism of action can be postulated. It must be noted that the response to individual components is assumed to be consistent with that occurring in a complex food matrix. Whether this is true or not remains to be adequately verified.

2. CINNAMIC ACID

Cinnamic acid, chemically related to benzoic acid, is ubiquitous in plants and fruits, providing a natural protection against infections by pathogenic microorganisms. Products containing cinnamon oil are particularly rich sources of cinnamic acid. Its relatively low toxicity coupled with its flavoring characteristics has fostered its commercial use *(36)*.

Cinnamic acid and associated derivatives possess a broad spectrum of antifungal and antibacterial activities *(36–38)*. Cinnamic acid decreases revertants in eukaryotic murine FM3A cells induced by ethyl methanesulfonate (an alkylating agent), hydrogen peroxide (an oxidizing agent), and quinacrine (a frameshift mutagen) *(39)*. Additionally, cinnamic acid has antitumor activity against a wide range of neoplasms *(40,41)*. Cinnamic acid may exert its antiproliferative effects by inhibition of protein isoprenylation, which in turn inhibits mitogenic signal transduction *(40,42)*. Likewise, cinnamoyl analogs are recognized to act as specific protein tyrosine kinase inhibitors, also possibly accounting for their ability to suppress tumor cell growth *(43)*. It must be emphasized that the concentrations of cinnamic acid necessary to bring about antitumorigenic properties are rather massive (2–8 m*M*). Thus, the true physiological importance in modifying cancer risk remains to be determined. Interestingly, the combination of interferon-α and cinnamic acid was more effective in retarding the proliferation of A549 human lung cancer than either compound alone *(44)*. Additional studies are needed to determine what effects, if any, cinnamic acid and associated compounds have on other models of cancer, especially those where dietary manipulation is possible.

3. FLAVONOIDS

The flavonoids are a group of organic molecules ubiquitously distributed in vascular plants. Approximately 4000 flavonoid compounds are known to exist. Typical dietary intakes of flavonoids are not known with any certainty but are estimated to be several hundred milligrams per day *(45)*. As modified phenolic compounds, these compounds can act as potent antioxidants and metal chelators. The cytochrome P450 (CYP) 1A

Table 2
Examples of Classes of Flavonoids Found in the Food Supply

Class	*Food sources*
Flavones	
Tangeretin	Citrus
Nobiletin	Citrus
Flavonols	
Quercetin	Fruits, vegetables, cereal grains
Kaempferol	Fruits, vegetables
Catechins	Tea
Flavanones	
Naringenin	Grapefruit
Isoflavones	
Genistein	Soybeans
Daidzein	Soybeans

enzyme appears to be predominately involved in flavonoid hydroxylation, whereas other cytochrome CYP enzymes are involved in their demethylation *(46)*. Overall, several of these flavonoids appear to be effective antipromoters of the cancer process. In addition, some appear to be effective inhibitors of the bioactivation of selected carcinogens. However, depending on the flavonoid as well as the specific foreign compound, the influence can sometimes be stimulatory, whereas in other cases it is inhibitory *(47)*.

Flavonoids are generally classified on the basis of substitutions occurring on one or more of the rings. These classes include flavones, flavonols, flavanones, and isoflavones. Table 2 lists some compounds within each class that have been examined for their anticarcinogenic properties.

3.1. Flavones

Tangeretin and nobiletin are two polymethoxylated flavonoids in citrus foods that have been examined for their anticarcinogenic properties *(48,49)*. These unhydroxylated compounds have been shown to modify several CYP enzymes. Canivenc-Lavier et al. *(50)* reported that tangeretin increased rat liver CYP 1A1, 1A2, and 2B1, 2 by about 80% and sixfold when provided at 0.3% in the diet for 2 wk. The ability of tangeretin to inhibit unscheduled DNA synthesis induced in cultured human liver slices by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-acetylaminofluorene (2-AAF) may be attributable to the these CYP changes *(51)*. Nevertheless, it remains to be determined how important these changes are in vivo in determining tumor incidence.

Both nobiletin and tangeretin are effective in inhibiting the growth of a variety of neoplastic cells in culture *(52,53)*. Both markedly inhibited the proliferation of a squamous cell carcinoma and a gliosarcoma cell line when added to the medium at relatively high concentrations (2–8 mg/mL) *(52)*. Tangeretin appears to bring about part of its antitumorigenic effects by causing G1 arrest and by decreasing the degree of phosphorylation of the retinoblastoma tumor suppressor protein *(49)*. The decrease in Cdk2 and Cdk4 or the increase of the Cdk inhibitors p21 and p27 may account for some of the signal changes involved with the G1 arrest *(49)*.

Some cells, such as the human lung fibroblast-like cell line (CCL 135), were relatively resistant to these flavonoids, pointing to the error in assuming that all tumors are identical in their sensitivity to these bioactive food components. Kawaii et al. *(53)* provided evidence that the orthocatechol moiety in ring B and a C2–C3 double bond were important for the antiproliferative activity. More recently, Bracke et al. *(54)* found that although tamoxifen and tangeretin exhibit similar inhibitory effects on the growth and invasive properties of human mammary cancer cells in vitro, only tamoxifen was effective in a xenograph transplant model, suggesting that absorption may be different between the two compounds. However, of greater concern was that when the two were combined, an interference with the effectiveness of tamoxifen emerged. Clearly, such evidence reveals that it is unwise, and possibly counterproductive, to use flavonoids as adjuvant therapy until their true impact on classical therapies are better understood.

3.2. Flavonols

3.2.1. Quercetin and Kaempferol

Although two of the most common flavonols—quercetin (3,5,7,3′,4′-pentahydroxyflavone) and kaempferol (3,5,7,4′-tetrahydroxyflavone)—do exhibit some mutagenicity in the Ames assay *(55)*, they also appear to be effective modifiers of cancer risk. Conjugates including *quercitrin* (quercetin-3-L-rhamnoside), quercetin-3-glucoside, and rutin (quercetin-3-β-D-rutinoside) are known to occur in nature and, at times, have biological activity. Flavonoids such as quercetin, rather than vitamin C, can contribute significantly to the total antioxidant capacity of fruits such as apples. In fact, quercetin contributes about four times as much antioxidant power as vitamin C in several cultivars of apples including Golden Delicious, Cortland, Monroe, Rhode Island Greening, and Empire *(56)*. Although flavonols can be absorbed into the bloodstream principally as glucosides, minor structural differences can markedly influence both the level of accumulation and the extent to which the conjugates are excreted *(57)*.

Quercetin is recognized as a potent modifier of CYP 1A1 reactions *(58)*. In studies by Verma et al. *(59)*, up to 5% quercetin was fed without apparent ill consequences to the rat and resulted in significantly reduced incidence of both 7,12-dimethylbenz[a]-anthracene (DMBA)- and *N*-methyl-*N*-nitrosourea (MNU)-induced mammary tumors *(59)*. Such data suggest that bioactivation is not essential for the protection provided. Evidence exists that quercetin can inhibit the promotion phase of DMBA- and MNU-induced skin tumors *(60)*. Similarly, considerable evidence points to the ability of quercetin and related compounds to modify the proliferation of existing neoplasms. These growth-inhibitory effects frequently coincide with a block in the G0/G1 phase of the cell cycle. Ranelletti et al. *(61)* found that quercetin addition to cultures of colon cancer cell lines produced a dose-dependent, reversible growth inhibition and that quercetin reduced p21-ras proteins by about 50% in both cultured colon cancer cell lines and primary colorectal tumors *(61,62)*. Also, it inhibited the expression of K-, H-, and N-ras proteins. More recently, the G1 arrest caused by quercetin in primary human foreskin keratinocytes in G1 was linked to an elevation in the cyclin-dependent kinase (cdk) inhibitor p27(Kip1). The elevation in p27 appeared to result from not only an increased half-life of the protein but also an increase in its messenger RNA (mRNA) transcription *(63)*. Moreover, evidence suggests that the antimitotic effect of quercetin may be caused by inhibition of microtubule polymerization resulting from direct binding of quercetin with tubulin protein *(64)*.

Quercetin has been shown to induce mitotic cell cycle arrest and trigger apoptosis in human breast cancer cells *(65)*. In human promyelocytic leukemia HL-60 cells, apoptosis induced by adding quercetin correlated with a loss of mitochondrial transmembrane potential, cytochrome c release, and caspase-9 and -3 activation *(66,67)*. Recent evidence suggests that this effect might involve the CD95 apoptotic pathway. This pathway has been well-characterized because it plays a fundamental role in immunosurveillance and in immune evasion by tumors. Fas antigen CD95 (Fas/APO-1) has become best known as a death receptor that activates the extrinsic apoptosis pathway. CD95 is a member of the tumor necrosis factor growth factor receptor superfamily and is currently recognized as the principal cell surface receptor involved in the signal transduction that induces apoptosis in the immune system and various tumor cells. Recently, quercetin was found in human pre-B (HPB)-acute lymphoblastic leukemia cells, which are normally resistant to CD95-mediated apoptosis, and sensitized this cell line through a mechanism independent of its antioxidant properties and likely through the activation of protein kinase C-α*(68)*.

Estrogenic potency of some flavonoids can be significant, especially for estrogen receptor (ER)-β. Thus, hormonal changes may trigger some of the biological responses that are evoked by some flavonoids *(69)*. Transcriptional activity experiments with nuclear ERs reveal a stronger activation of ER-β than ER-α by genistein *(69)*. However, genistein and the flavonid quercetin have also been shown to activate endogenous ER-α in human breast cancer cells *(70)*. Interestingly, at concentrations that are sufficient to promote transcriptional activity they simulate proliferation; however, at higher concentrations these phytoestrogens are strong cytotoxic agents that can kill ER-independent HeLa cells *(70)*. Overall, the mode of action of phytoestrogens and the balance between either risk or chemopreventive factors for breast cancer may depend heavily on the total dietary intake. Likewise, it must be emphasized that not all tumors are equally sensitive to flavonols, and those that are sensitive vary considerably. *(52)*.

3.2.2. Green and Black Tea Flavonols

Grown in about 30 countries, tea is consumed with considerable variability as a beverage among individuals *(71)*. Although tea continues to be the most widely consumed beverage (other than water), consumption is only about 0.12 L/yr. Tea is manufactured in three basic forms: unoxidized (green) tea, oxidized (black) tea, and partially oxidized (oolong) tea. Only about 20% of the tea produced is green, whereas less than 2% is oolong. Green tea is consumed primarily in China, Japan, and a few countries in North Africa and the Middle East, although it is gaining in popularity in other parts of the world.

Fresh tea leaves are unusually rich in polyphenols. These catechin polyphenolics may constitute up to 30% of the dry leaf weight. Other polyphenols include flavonols and their glycosides, chlorogenic acid, coumarylquinic acid, and one unique to tea—theogallin (3-galloylquinic acid). Various quinones are produced by oxidation and condense to form a series of compounds, including bisflavanols, theaflavins, epitheaflavic acids, and thearubigens, which give rise to the characteristic taste and color properties of black tea *(71)*. Green tea composition is very similar to that of the fresh leaf except for a few enzymatically catalyzed changes that occur following harvesting. Thearubigens constitute the largest mass of the extractable matter in black tea. Oolong tea is intermediate in composition between green and black teas.

Although inconsistencies exist, some human consumption of tea or tea extracts has been associated with a reduction in cancer *(72–79)*. In particular, there may be benefits

for digestive tract cancers, although intakes of tea associated with a protective effect are high *(72)*. It should be noted that exaggerated intakes may also create side effects in some individuals *(80)*. Unexpectedly, Lu et al. *(81)* suggested that tea consumption is associated with an increased risk of bladder cancer. Discrepancies among studies may result from confounding of other lifestyle factors such as smoking and alcohol consumption *(73)*, inadequate characterization of tea consumption and preparation differences *(72,82,83)*, and individual differences in metabolism of bioactive tea constituents *(73)*. Obviously, additional attention is needed to determine if, and under what circumstances, tea consumption may modify one or more phases of the cancer process. It should be highlighted that considerable attention has recently focused on the use of tea polyphenolics in skin cancer chemoprevention strategies *(84–88)*. Also, tea polyphenols may ultimately find greater use for cancer prevention in combination with other phytochemicals or drugs *(83,89,90)*.

Recently, substantial progress has been made in understanding the human complexities of tea polyphenolic bioavailability and pharmacokinetics in humans. In general, tea polyphenols are poorly bioavailable and extensively metabolized by colonic microflora *(73,91,92)*. Because phenolic metabolites may have less antioxidant activity than their parent compounds, care should be taken in the interpretation of in vitro findings, especially those examining nonphysiological levels of phenolic compounds. Tea catechins differ considerably in their pharmacokinetic behavior and metabolism when compared to conjugated and methylated forms *(93–95)*. This may partially explain the reason that the magnitude of increase in plasma antioxidant activity in humans following ingestion of tea polyphenolics varies and the reason that plasma antioxidant strength can be maintained only by continuous daily intakes *(93,96–98)*.

In contrast to human data, a variety of animal model bioassays have consistently found that treatment with the polyphenolic fraction isolated from green tea leaves protects against chemically induced cancers. Chemically induced tumors in the large intestine, forestomach, liver, skin, lung, pancreas, and mammary tissue all have been reported to be suppressed by exaggerated exposure to tea or associated polyphenolics *(72,73,99,100)*.

The chemopreventive effects of green tea may be attributed to several polyphenolic compounds, particularly the catechins epigallocatechin-3-gallate (EGCG), epigallocatechin, and epicatechin-3-gallate, which account for 30 to 40% of the extractable solids of tea leaves. It is likely that several mechanisms of action may contribute to the observed anticancer effects of tea constituents, including serving as a free-radical scavenger or antioxidant, inducing cell cycle arrest and detoxification of carcinogens through altered phase I and II enzymes, modulating inflammation-associated eicosanoid metabolism, and stimulating apoptosis *(32,101,102)*.

Carcinogen detoxification is a key event in preventing the initiation of chemical carcinogenesis. Tea polyphenols have been reported to inhibit DNA damage resulting from polycyclic aromatic hydrocarbons and heterocyclic amines, effects mediated in part by enhancing carcinogen degradation and suppressing mRNA abundance of CYP 1A1 and CYP 1B1 bioactivating enzymes *(103,104)*. Shi et al. *(105)* found that EGCG inhibited the catalytic activities of several CYP isozymes and was more potent against CYP 1A and 2B1 than it was against 2E1. Several tea preparations have been reported to induce phase II enzymes such as glutathione-*S*-transferase (GST) *(106,107)*, an effect that may depend on involvement of gut bacterial metabolites *(108)*. Several tea preparations can

inhibit nitrosamine formation *(109)*. Because the vast majority of nitrosamines are known carcinogens, at least in animal models, these studies may have particular significance *(110)*. Wu et al. *(111)* demonstrated that the amount of the hepatocarcinogen *N*-nitrosomorpholine (NNM) formed in vitro depended on the molecular structure of tea catechin derivatives and their molar ratios to nitrite. Therefore, the depression in nitrosamine formation relates to a sequestering of nitrite, making it unavailable for the formation of the carcinogen. A small human study confirmed that tea supplementation can significantly suppress fecal nitrite *(112)*. Not only can various tea preparations decrease the formation of nitrosamines, they also can depress their bioactivation. Various teas have been reported to block nitrosamine-induced tumorigenesis in skin *(113)*. Thus, a reduction in carcinogen exposure may partially account for some, but not all, of the protection provided by supplemental tea and its polyphenols under experimental conditions.

In addition to their capacity to prevent early initiating events in carcinogenesis, tea phenolics may act to modify metabolic pathways contributing to the promotion/progression of cancers. In preclinical studies, tea polyphenols have been observed to exhibit antioxidant, anti-inflammatory, antiproliferative, proapoptotic, and antiangiogenic actions *(114–124)*. The mechanisms underlying these effects are multiple, including modulation of telomerase activity, altering the balance of signal transduction pathways, changing the metabolism of arachidonic acid, and enhancing free-radical scavenging capacities. Recent evidence suggests that tea polyphenols may differentially affect neoplastic and normal cells *(125,126)*. An intriguing report recently demonstrated a reversal of the multidrug resistance phenotype by tea polyphenols *(127)*, a finding that, if confirmed, could open up new strategies for chemotherapy of some tumors.

Collectively, considerable information supports a preventative role of green and black tea against each stage of carcinogenesis. The mechanisms by which these tea components afford these diverse effects appear to be multifold, with the most notable being an alteration in both phase I and II enzymes and alterations in cell signaling. Confirmation of the preclinical data with human intervention trial data is sorely needed.

3.3. Flavanones in Grapefruit

Naringin is the most abundant flavonoid in grapefruit. It has been shown to inhibit the activation of aflatoxin B_1 *(128)* but was relatively ineffective in blocking unscheduled DNA synthesis in liver slices exposed to PhIP *(51)*. Part of the modest protection provided by naringin against cancer models may relate to its ability to induce phase II enzymes. These changes can have unexpected consequences, as evident by the ability of grapefruit juice to markedly augment oral drug bioavailability. The ability of naringin and other compounds in grapefruit to downregulate CYP 3A4 expression posttranslationally serves to illustrate important drug–nutrient interactions that can occur *(129)*. Actually, several compounds in grapefruit may account for the depression in CYP 3A4 activity *(129)*. It will become increasingly important as fruit and vegetable consumption (hopefully) increases to understand their impact on a host of drugs that may be used for various health conditions.

3.4. Isoflavones in Soybeans

Considerable evidence points to the ability of isoflavonoids in soybeans to alter the cancer process *(130,131)*. Phytoestrogens present in the soybean and other legumes

have been purported to contribute to the international differences in cancer prevalence, most notably that of the breast. However, epidemiological studies indicate that a protective effect of adult soy intake on breast cancer risk is modest at best and hint that the age at consumption is a key factor in determining response *(132–135)*. Compared to several other foods, soybeans supply relatively large amounts of four different types of compounds that may have anticarcinogenic properties: glycosides, phytosterols, protease inhibitors, and phytic acid. Soybeans are known to comprise about 2% glycosides, which are composed of soya saponins and isoflavonoids. Plant isoflavonoid glycosides generally are converted by intestinal bacteria to form hormone-like compounds with weak estrogenic and antioxidative activity.

Much of the attention of soybean isoflavonoids has been directed at daidzein, equol, and genistein. Although soybean isoflavones are weak estrogens, they can function both as estrogen agonists and antagonists depending on the hormonal milieu, the target tissue, the species being examined, and the amount consumed. Evidence now points to the ability of these compounds to influence not only sex hormone metabolism and associated biological activity but also intracellular enzymes, protein synthesis, growth factors, malignant cell proliferation, and differentiation *(136–138)*.

Considerable evidence points to the ability of isoflavonoids to inhibit chemically induced carcinogenesis. Part of this response may relate to changes in selected CYP enzymes. For example, the isoflavones genistin and daidzin, and their respective aglycone forms daidzein and genistein, were found to block 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced CYP 1A1 enzyme activity. The response was independent of aromatic hydrocarbon receptor but appeared to involve a noncompetitive inhibition with the CYP 1A1 substrate *(139)*.

Genistein also has attracted considerable attention because of its ability to inhibit several enzymes, including protein tyrosine kinases and others involved in signal transduction *(140)*. Genistein can attenuate both growth factor- and cytokine-stimulated proliferation of both normal and neoplastic cells. Although genistein is a potent inhibitor of tyrosine autophosphorylation of the epidermal growth factor receptor, this response does not coincide with a suppression in cell growth, suggesting the involvement of other signal transduction pathways as possible sites of action. The alternative sites include a retarding of DNA topoisomerase II activity, a variable inhibition of cell cycle checkpoints, and/or antiangiogenic activity *(140,141)*. Genistein also may inhibit cell growth by modulating transforming growth factor (TGF)-β-1 signaling pathways *(140)*.

Lamartiniere et al. *(142)* provided convincing evidence that neonatal and prepubertal exposure to genistein has a long-lasting effect on the ability of the mammary gland to withstand exposure to carcinogen. Part of this protection may relate to a reduction in the number of carcinogen-sensitive terminal end buds and an increase in the number of differentiated, carcinogen-insensitive lobular structures within mammary tissue. However, soy and genistein may also increase the risk of some cancers, especially those that are estrogen-sensitive *(143)*. Although experimental data are rather compelling that genistein can induce the growth of human mammary tumors transplanted into nude mice *(144)*, it remains to be determined if this coincides with a more aggressive tumor that is more invasive to surrounding tissue or one that metastasizes to a greater degree. It may be that other metabolic changes induced by soy, genistein, or equol outweigh this change in estrogen-sensitive tumor growth. However, it would be unwise and irresponsible to dismiss this as a simple experimental phenomena with no physiological significance. It

may be that some women will be placed at risk by exaggerated intakes of soy and its components.

Human intervention studies provide inconsistent evidence for a protective effect of soy intake on biomarkers of breast cancer susceptibility, such as modulation of estrogen metabolism. Studies in both pre- and postmenopausal women indicate that soy or isoflavone consumption has a beneficial effect, although the magnitude is small *(145–147)*. Although soy and isoflavone intakes do not seem to affect the endometrium in premenopausal women, there is some concern about a possible weak estrogenic effect in the breast, as evidenced by higher secretion of breast fluid and the appearance of hyperplastic epithelial cells and elevated levels of pS2 in the nipple aspirate *(148,149)*. However, when considering mammographic breast density as a biomarker of breast cancer risk, soy or isoflavone intake does not appear to exert an estrogenic effect similar to that of hormone replacement therapy and may have a beneficial influence *(150–155)*.

Although considerable attention has focused on the breast cancer-modifying action of soy, there has been a recent upsurge in interest in soy for prostate cancer prevention *(156)*. Reduced risk for prostate cancer has been reported in Chinese men consuming higher levels of soy foods and isoflavones *(157)*. Cell culture and animal model experiments have confirmed a prostate cancer-suppressing effect of soy and genistein, an effect mediated in part by changes in androgen metabolism, decreases in insulin-like growth factor (IGF)-1-stimulated cell proliferation, and increased apoptosis *(158–162)*. Therefore, the use of soy isoflavones in prostate cancer prevention trials may be justified *(163,164)*.

Vasculature has an important role in several steps of the cancer metastatic process including the following: (a) providing access to distant sites of metastasis, because vessels capture cancerous cells and provide the entry route into secondary organs, and (b) angiogenesis, because vascular endothelial cells supply nutrients for tumor growth. The linings of all blood vessels are covered with endothelial cells, which can have an active role in both processes. Several studies have provided evidence that the consumption of plant foods can prevent or retard angiogenesis or neovascularization. Soy products and genistein appear to be potent inhibitors of endothelial cell proliferation and in vitro angiogenesis *(165)*. Degradation of the extracellular matrix is one of the essential steps in angiogenesis. Studies by Fajardo et al. *(166)* demonstrated that genistein could induce a shift toward antiproteolysis in both matrix metalloproteinase/tissue inhibitor of metalloproteinase and urokinase/plasminogen activator inhibitor proteolytic balance. Overall, the anticancer effects of soy may relate to a direct effect on tumor cells as well as indirect effects on tumor neovasculature.

4. INDOLES

Several indole metabolites arise during the ingestion and the hydrolysis of the indolylmethyl glucosinolate glucobrassicin that is present in cabbage, broccoli, Brussels sprouts, and other members of the genus *Brassica (167)*. The resultant products are numerous and include indole-3-carbinol (I3C). I3C is unstable in the acid environment of the stomach and can undergo self-condensation reactions to form indole [3,2-b]carbazole (ICZ) and diindoylmethane (DIM) as well as other trimers and oligomers *(168,169)*. DIM is the major intermediate formed in vitro and in vivo. Preclinical studies

indicate that I3C has preventive activity against numerous cancers, including those of the mammary gland, prostate, stomach, lung, liver, endometrium, and colon; however, the effects may not always be beneficial *(170)*. Although many experimental cancer studies have used rather large quantities of I3C (0.5–3%), providing as little as 56 mg/kg is sufficient to alter enzymes involved in carcinogen bioactivation *(171)*. Thus, it is conceivable that changes in cancer risk may occur with very modest intakes. The protection provided likely depends on the species and tissue examined, although humans do appear to be sensitive to I3C intakes *(172,173)*. Several mechanisms have been elucidated whereby I3C and related indoles may modify carcinogenesis at both the initiation and postinitiation stages. These include modifying the endogenous metabolism of estrogens, stimulating apoptosis, augmenting cellular defenses against genotoxic chemicals, and modifying cellular signaling pathways that control proliferation.

To date, the effects of I3C on enzymes associated with estrogen and xenobiotic metabolism have been studied extensively. Particular attention has been given to the phase I CYP 1A1, 1A2, and 3A4 enzymes. Whereas both I3C and DIM can induce these three enzymes in short-term studies, after chronic exposure, I3C is more efficacious than DIM *(174)*. DIM also decreases CYP 1B1, depresses 4-hydroxylation of estradiol, and decreases carcinogenic 4-OH-estrone *(175)*. The effectiveness of I3C as an inducer is dependent on the activation by its condensation products ICZ and DIM of the aryl hydrocarbon receptor (AhR) *(176–178)*. Such changes in CYP-mediated oxidative metabolism of estrogen are believed to be responsible for not only carcinogen detoxification (discussed later) but also for the indole-induced increase in the ratio of 2-OH-estrone to 16-OH-estrone, which is hypothesized to be protective toward breast cancer *(179)*. The ability of oral I3C to enhance C-2 hydroxylation while depressing that of C-16 is readily apparent in females from several species *(172,180)*. In animal models, modulation of CYP activity and estrogen metabolism are dose-dependent, and treatment is duration-dependent. It should be noted that genetic polymorphisms might impact the actions of indoles. The presence of a novel Msp1 restriction fragment length polymorphism of CYP 1A1 in African-American, but not Caucasian, women modified the magnitude of the 2-OH-estrone/16-OH-estrone ratio following I3C administration *(181)*.

Besides its effects on estrogen metabolism, I3C exposure also may alter the formation of genotoxic intermediates that could initiate carcinogenesis. This response may arise because of changes in both phase I and II enzymes. For example, I3C has been shown to reduce DNA adduct formation and reduce γ-glutamyl transpeptidase activity in mouse skin induced by DMBA and benzo(a)pyrene *(182,183)*. Likewise, I3C has been shown to decrease the binding of PhIP and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) to mammary tissue *(184)*. This response is particularly intriguing because both are recognized carcinogenic heterocyclic amines formed in proteinaceous foods during cooking and are known to be initially activated enzymatically by CYP 1A1 and 1A2. In addition to the changes in phase I CYP enzyme activities noted earlier, consumption of I3C alters the activities of several phase II enzymes including glutathione *S*-transferase, quinone reductase ,and glutathione reductase *(171,185)*. Although in general I3C and DIM appear to enhance detoxification of chemical carcinogens, the ultimate balance between a decrease or increase in carcinogen activation and DNA damage will depend on the specific indole, its dose, the carcinogen examined, and the target tissue *(170,186–188)*. For example, in contrast to high doses of I3C given to male rats, low

doses shifted the ratio of hepatic CYP 1A2 vs CYP 1A1 such that there could be an increase in activated IQ *(188)*.

Evidence exists that I3C can influence both the promotion and progression phases of some chemically induced cancers in addition to its actions during the initiation stage described earlier. I3C may affect cell proliferation not only by indirectly altering estrogen metabolism but also by directly blocking the cell cycle and inducing apoptosis. It has been reported that exposure of mammary epithelial cells to I3C can decrease nuclear ER and progesterone receptor binding and inhibit estrogen-stimulated cell proliferation *(189,190)*. I3C may modify the activation and subsequent signaling events of the ER *(191–194)*, in some cases in a ligand-independent manner *(192)*. Additionally, an indole-induced antiproliferative signaling pathway has been reported in breast cancer cells that leads to G1 cell cycle arrest. This arrest resulted in part from decreased cdk6 activity and a concomitant increase in expression of the cdk inhibitor $p21^{waf1/cip1}$ *(195)*. It is clear that dissecting the complex interactions by which indoles alter cancer cell multiplication warrants further scrutiny.

The ability of I3C to inhibit virally induced tumors comes from studies showing that it inhibits the spontaneous occurrence of endometrial adenocarcinoma in female Donryu rats and, more recently, from several studies with virally induced cervical cancer *(196,197)*. Estrogen is known to promote the development of endometrial and cervical cancers. Jin et al. *(197)* provided evidence that I3C may be particularly useful in the prevention of cervical-vaginal cancer, and possibly other cancers with a papillomavirus component. Studies by Yuan et al. *(198)* with cervical CaSki cancer cells provided evidence that I3C can abrogate estrogen-increased expression of cells infected with human papillomavirus oncogenes, probably by enhancing the formation of 2-hydroxyestrone by the mechanism indicated earlier. In this regard, it is important to highlight that treatment with I3C of individuals with precancerous lesions of the cervix has been effective in human translational studies *(199–201)*.

A depression in cell proliferation also may arise from the induction of apoptosis. There is substantial evidence that I3C and related indoles are capable of stimulating apoptosis in a variety of cancerous cells *(202–207)*, an effect apparently not observed in indole-treated noncancerous cells *(204)*. I3C induction of apoptosis in noncancerous human mammary epithelial 184-B5 cells was accompanied by enhanced p53 immunoreactivity *(205)*.

Consumption of indoles does not always result in a beneficial effect on tumorigenesis. Increased tumorigenesis has been reported for colon, liver, thyroid, and pancreas *(170)*. These studies suggest that the response to I3C is highly dependent on the timing of administration; postcarcinogen treatment with I3C often leads to promotion of tumorigenesis. The mechanisms for these enhancing effects remain to be determined, although a recent report provides evidence for a tumor-promoting mechanism in that treatment of rat hepatocytes with ICZ resulted in an inhibition of gap junctional intercellular communication associated with activation of the AhR and/or CYP 1A activity *(208)*.

Collectively, considerable evidence points to the health benefits associated with enhanced I3C consumption. However, because some cases of enhanced carcinogenesis are evident, it is prudent that we understand who might be placed at risk before widespread recommendations for increased intakes of I3C are encouraged *(170,209)*.

5. ISOTHIOCYANATES

Cruciferous vegetables (e.g., broccoli, Brussels sprouts, cabbage, cauliflower, kale, etc.) belong to the genus *Brassica* and possess a variety of sulfur-containing glycoside precursors (or glucosinolates) that yield thiocyanate, nitrile, and isothiocyanate derivatives upon hydrolysis and release of a glucose moiety. This hydrolysis is carried out by the enzyme myrosinase, which is released from plant cells damaged by food processing and mastication *(210,211)*. The chemopreventive benefit of cruciferous vegetable consumption has been attributed in part to the naturally occurring isothiocyanates present within these foods *(212)*. Myrosinase is notably heat-labile, and cooking broccoli has been shown to strongly inhibit isothiocyanate bioavailability in humans *(213,214)*. Isothiocyanates such as phenethyl isothiocyanate (PEITC), benzyl isothiocyanate (BITC), and sulforaphane have received much attention recently, as increasing evidence suggests these compounds may act as key inhibitors of carcinogenesis in a variety of tissues including liver, lung, mammary gland, intestine, forestomach, and esophagus *(215–219)*.

Organic isothiocyanates have been reported to block tumor formation in rodents induced by such diverse carcinogens as polycyclic aromotic hydrocarbons, azo dyes, ethionine, *N*-2-fluorenylacetamide, and nitrosamines *(215)*. Substantial evidence obtained from studies of lung tumorigenesis in rodent models indicates a protective effect of selected isothiocyanates against the tobacco smoke-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) *(220–222)*. Nevertheless, isothiocyanates do not always provide protection. In some cases, they may actually accentuate tumor development. For example, Hirose et al. *(223)* found that PEITC and BITC promoted the postinitiation phase of diethylnitrosamine-induced bladder carcinogenesis. Similarly, Stoner et al. *(224)* found that dietary 6-phenylhexylisothiocyanate promoted *N*-nitrosomethylbenzylamine-induced esophageal carcinogenesis.

Several isothiocyanates appear to be effective inhibitors of carcinogen metabolism, as evidenced by a reduction in carcinogen binding to DNA in target tissue *(215,216,225)*. This inhibitory effect on the initiation stage of carcinogenesis appears to be mediated via induction of phase II detoxification enzymes, including glutathione transferases and NAD(P)H:(quinone acceptor) oxidoreductase, and suppression of cytochrome CYP-mediated carcinogen activation *(215,216)*. CYP 1A2 is known to catalyze bioactivation of the nitrosamine lung carcinogen NNK, and PEITC has been shown to competitively inhibit CYP 1A2 activity *(226)*. Similarly, the nitrosamine *N*-nitrosodimethylamine is bioactivated via CYP 2E1, the activity of which is inhibited by PEITC, BITC, and sulforaphane *(227–229)*. Inactivation of CYP 2E1 in response to BITC may relate to its modification of the CYP apoprotein *(227)*. Sulforaphane, which is both a potent competitive inhibitor of CYP 2E1 and an inducer of phase II detoxification enzymes, has been shown to block mammary tumor formation in rats dosed with 9,10-dimethyl-1,2-benzanthracene *(229,230)*.

Isothiocyanates may also modify the cancer process by inducing apoptosis and suppressing metastasis. Early studies revealed that isothiocyanic esters inhibited the in vivo growth of transplanted Ehrlich ascites carcinoma cells *(231)*. Zhang et al. *(232)* recently revealed that short-term exposure to several isothiocyanates was sufficient to suppress cell proliferation, even in some drug-resistant cell lines. Part of the depression in growth is also likely attributable to induction of apoptosis. PEITC has been

found to induce p53 protein expression and p53-dependent transactivation. The significance of these findings is also evident by the ability of PEITC to induce apoptosis in p53 +/+, but not p53 −/−, cells *(233)*. Exposure of human colon cancer cells (HT29 cells) to sulforaphane has been shown to trigger apoptosis; however, this result appears secondary to an arrest of the cell cycle at G_2/M. Sulforaphane-induced HT29 cell death, although characterized by molecular and morphological apoptotic markers, was not associated with an effect on p53 expression *(234)*. In addition, Sasaki et al. *(235)* demonstrated that oral administration of 5-methylthiopentyl isothiocyanate markedly reduces the pulmonary colonization of B16-F10 murine melanoma cells in syngeneic mice. This protection was not without some complications, as it also resulted in thymus atrophy and a selective loss of $CD4^+CD8^+$ cells in thymocytes. Interestingly, neither the metastaticity or thymus responses were observed in mice treated with 3-methylthiopropyl isothiocyanate.

The protective effect of isothiocyanates may be highly dependent on its clearance within an individual or possibly within a specific tissue. Glutathione transferase enzymes are known to conjugate isothiocyanates, leading to their excretion, which largely occurs as dithiocarbamates *(213)*. Recent evidence suggests that individuals with a glutathione transferase M1 null genotype may have a more exaggerated response to broccoli and associated isothiocyanates *(236)*.

6. ORGANOSULFUR COMPOUNDS

Garlic, onions, leeks, and chives represent the major *Allium* foods consumed by humans. Considerable evidence points to their consumption, particularly garlic, as possible modifiers of cancer risk. In recent reviews of over 35 case–control or cohort studies, it was reported that some protective effect was associated with intake of *Allium* vegetables in about 75% of the studies *(237–240)*. The strongest evidence was for stomach and colorectal cancers. Data for a protective effect toward prostate cancer are limited, although a recent case-control study conducted in China *(241)* reported a significant 49% reduction in risk for those men with the highest *Allium* vegetable intakes compared to those in the lowest intake category, an effect more pronounced for subjects with localized cancer than for those with advanced lesions. Data linking garlic intake and precancerous lesions are also limited, although one large case–control study reported lower risk of colorectal polyps with increased garlic consumption *(237)*. On the other hand, the evidence for a cancer-protective benefit of garlic *supplement* intake is limited and inconsistent *(237,242)*. A large, unpublished trial is being conducted in China to determine the effects of two garlic preparations on precancerous lesions of the stomach *(243)*. Unfortunately, inadequate information is provided far too frequently about the form and quantity of the garlic preparation provided and consumed *(237)*. In addition to breath and body odors, adverse consequences associated with a large amount of garlic intake include esophageal and abdominal pain and increased blood coagulation times *(237,243)*. The influence on blood coagulation is one reason that concerns have been expressed about garlic and aspirin interactions. Garlic consumption also may influence drug metabolism and, therefore, influence their serum concentrations, such as with saquinavir (Fortovase, Invirase), and potentially influence its therapeutic efficacy *(244,245)*. These effects need to be more carefully documented, especially those associated with specific types of formulations *(237,242)*.

Table 3
Some of the Important Sulfur Compounds Found in Garlic

3,5-Diethyl-1,2,4-trithiolane	Diallyl sulfide
Allyl 1-propenyl disulfide	Diallyl trisulfide
Allyl 1-propenyl trisulfide	Methyl allyl trisulfide
Allyl alcohol	Methyl allyl disulfide
Diallyl disulfide	*S*-allyl cysteine

Unlike many other foods, about 0.35% of garlic's fresh weight or 1% of its dry weight is contributed by sulfur *(246,247)*. A complex array of sulfur compounds (Table 3), including thiosulfinates, dithiins, ajoenes, etc., can occur in garlic preparations *(248–250)*.

Although major limitations exist in defining the precise role of garlic in the cancer process, laboratory-based studies with model cancers provide some of the most compelling evidence that garlic and its related sulfur components can suppress cancer risk and alter the biological behavior of tumors. Experimentally, garlic and its associated components suppress breast, colon, skin, uterine, oral, esophageal, and lung cancers *(238,251–253)*. This protection may arise from several mechanisms, including blockage of nitrosamine formation, free-radical scavenging, suppressed bioactivation of several carcinogens, enhanced DNA repair, decreased cell proliferation, and/or the induction of apoptosis. It is possible, and quite probable, that several of these cellular events are modified simultaneously.

Damage to DNA, proteins, and lipids by reactive oxygen species (ROS) is believed to be an important contributor to a variety of chronic diseases, including cancer. It has been suggested that garlic constituents can suppress oxygen radical-mediated cell damage by various mechanisms, including direct scavenging of ROS, stimulating formation of cellular and antioxidant defense compounds (such as glutathione), increasing enzyme activities (such as superoxide dismutase, catalase, and glutathione peroxidase), and blocking the activation or expression of stress pathways induced by ROS, such as NFκB *(254–256)*. In this regard, it has recently been demonstrated in a small human trial that dietary supplementation with a garlic extract for 2 wk substantially decreased plasma and urine concentrations of 8-iso-prostaglandin $F_{2\alpha}$, a sensitive and specific indicator of oxidative stress in vivo *(257)*.

As mentioned earlier, nitrosamines are potent experimental carcinogens. Mounting evidence demonstrates the ability of several foods, including garlic, to suppress their formation both in vitro and in vivo *(258–260)*. Garlic appears to be effective in blocking DNA alkylation, a primary step in nitrosamine carcinogenesis *(261,262)*. A block in nitrosamine bioactivation may reflect changes in several enzymes. However, substantial evidence points to the involvement of CYP 2E1 *(262,263)*. An autocatalytic destruction of CYP 2E1 may account for some of the chemoprotective effects of diallyl sulfide,and other possible allyl sulfur compounds *(264)*. The reduction in nitrosamines also may arise secondarily to an increase in the formation of nitrosothiols. Studies by Dion et al. *(259)* revealed that not all allyl sulfur compounds are equally effective. The ability of *S*-allyl cysteine and its non-allyl analog *S*-propyl cysteine—but not diallyl disulfide, dipropyl disulfide, and diallyl sulfide—to retard suggests that the cysteine residue is critical the block in nitrosamine formation *(259)*. Because the content of allyl sulfur can

vary among preparations, it is probable that garlic sources may vary in the protection they provide against nitrosamine formation. Evidence that garlic depresses nitrosamine formation in humans comes from studies by Mei et al. *(265)*. In their studies, providing 5 g of garlic per day completely blocked the enhanced urinary excretion of nitrosoproline caused by ingesting supplemental nitrate and proline.

Garlic and several of its allyl sulfur compounds can also effectively block the bioactivation and carcinogenicity of a host of non-nitrosamine carcinogens. For example, in rodent cancer models and in cell culture, garlic components have effectively suppressed the formation of DNA adducts, long considered to be a key early event in the initiation of carcinogenesis by chemicals *(244,266,267)*. This protection, which involves a diverse array of compounds and several target tissue sites, suggests multiple mechanisms of action or a widespread biological effect. Because metabolic activation is required for many of the carcinogens used in these studies, there is a likelihood that phase I and II enzymes are involved. Interestingly, little (if any) change in CYP 1A1, 1A2, 2B1, or 3A4 activities (enzymes involved in polycyclic aromatic hydrocarbon carcinogen bioactivation) is observed following treatment with garlic or related sulfur compounds *(268–270)*. Thus, other enzymes involved in the bioactivation or removal of carcinogenic metabolites might be involved in the observed protection.

Singh et al. *(271)* provided evidence that the efficacy of various organosulfides to suppress benzo(a)pyrene tumorigenesis correlated with their ability to induce NAD(P)H:quinone oxidoreductase, an enzyme involved with the removal of quinones associated with this carcinogen. Changes in bioactivation resulting from a block in cyclooxygenase and lipoxygenase may also partially account for the reduction in tumors following treatment with some carcinogens *(272)*. Also alterations in glutathione concentration and the activity of specific GST enzymes, important contributors to phase II detoxification, may be involved in the protection provided by garlic. Feeding garlic powder to rats has been found to increase GST activity *(273,274)*. Hu et al. *(275)* provided evidence that the induction of GST-pi may be particularly important.

Arylamine *N*-acetyltransferase (NAT) is an enzyme that can inactivate arylamines and activate some dietary heterocyclic amines. NAT polymorphisms affecting rates of acetylation have been linked to changes in cancer risk. Allyl sulfides have been reported to inhibit not only NAT activity in cell lines from human colon and bladder tumors *(244,276)* but also its activity in the bacterium *Helicobacter pylori* obtained from peptic ulcer patients *(277)*. Interestingly, garlic extract can decrease *H. pylori*-induced gastritis in Mongolian gerbils *(278)*, an effect of potential human application, because infection with this microbe is intimately involved in the development of stomach cancer.

Some allyl sulfur compounds are known to block cell growth in the G2/M stage of cell division *(279–281)* and also stimulate apoptotic events. Activation of the $p34^{cdc2}$ kinase complex is known to modulate the progression of cells from the G_2 into the M phase by promoting chromosome condensation, cytoskeletal reorganization, and nuclear envelope breakdown *(282)*. Recent studies by Milner et al. *(281,283)* revealed that DADS caused a marked suppression in $p34^{cdc2}$ kinase activity. Of recent interest is the report that DADS is able to effectively induce, via a p53-dependent mechanism, *NAG-1*, a gene that is also upregulated by other antitumorigenic and proapoptotic agents such as nonsteroidal anti-inflammatory compounds *(284)*. Also, an apoptosis-associated gene was among 14 genes identified in human cancer cells that were differentially expressed in response to diallyl trisulfide *(285)*.

Table 4
Some Important Sulfur Compounds Found in Onions

1–Propenyl propyl disulfide	Methyl 1–propenyl trisulfide
1–Propenyl propyl trisulfide	Methyl propyl disulfide
1–Propenyl methyl disulfide	Methyl propyl trisulfide
Dimethyl trisulfide	Dipropyl trisulfide
Dipropyl disulfide	
Dipropyl tetrasulfide	

Rarely has a comparison of water- and oil-soluble compounds been conducted within the same study. Although subtle differences among garlic preparations occur, quantity rather than source appears to be a key factor influencing the degree of protection *(286)*. Differences that do occur between preparations likely relate to the content and effectiveness of individual sulfur compounds. The number of sulfur atoms present in the molecule seems to influence the degree of protection with diallyl trisulfide > diallyl disulfide > diallyl sulfide *(279)*. Likewise, the presence of the allyl group generally enhances protection over that provided by the propyl moiety *(287)*.

6.1. Onions

Relatively few epidemiological and preclinical studies are available to truly assess the effect of onions on the cancer process. Nevertheless, an inverse relationship has been reported between lung, colorectal, and stomach cancer and the consumption of onions and leeks *(288–291)*. Recent investigations suggest that increased consumption of onions and other *Allium* foods is associated with a reduction in prostate cancer *(241)*.

Onions contain a variety of sulfur compounds, some of which are included in Table 4. Several of these have been examined for their ability to modify one or more stages of the cancer process.

Belman *(292)* reported in an animal model that onion oil inhibited skin tumor yield and incidence following phorbol-myristate-acetate promotion in a dose-dependent manner over the wide range when applied three times per week. Interestingly, onion oil was more effective than garlic oil in the inhibition of these tumors. More recently, Ito et al. *(293)* observed that diphenyl disulfide and *S*-methyl methanethiosulfonate significantly suppressed chromosomal aberrations induced by both aflatoxin B1 (an indirect-acting carcinogen) and methyl methanesulfonate (a direct-acting carcinogen). Changes in redox status of thiols were proposed as a possible mechanism of action. Clearly, additional studies are needed to examine if protection by onions depends on the carcinogen employed. Takada et al. *(294)* found that some sulfur compounds (isothiocyanic acid isobutyl ester, dipropyl trisulfide, and allyl mercaptan) promoted liver cell proliferation in rats treated with dimethylnitrosamine, suggesting that under some circumstances, the consumption of onions may increase cancer risk. The level of importance of these findings for humans remains to be determined, especially because Fukushima et al. *(295)* reported that oil-soluble organosulfur compounds such as methyl propyl disulfide and propylene sulfide as well as water-soluble compounds such as *S*-methylcysteine and cysteine retarded the development of GST-P-positive foci in the liver of rats treated with diethylnitrosamine.

The mechanisms by which onion components retard carcinogenesis remain largely unexplored. As indicated earlier with garlic, alterations in various biological processes

by selected thiols may account for part of the anticancer benefits associated with sulfur compounds. However, other constituents such as the flavanoids may account for the observed effects of onion as well as garlic on the cancer process. Regardless of the compounds responsible, extracts and essential oils of onions are known to have other physiological effects, including the retarding of platelet aggregation *(296)*. Interestingly, heating onions tends to reduce their efficacy.

Although the health benefits of onion consumption have not been extensively examined, available evidence does suggest there is little reason to reduce its use as part of a healthy diet. Nevertheless, the minimum quantity of onions needed to reduce cancer risk and the factors that might modify this efficacy remain to be determined.

7. TERPENES

Terpenes are compounds consisting of varying numbers of isoprene building blocks that are widely dispersed in nature. D-limonene is probably the most extensively examined monoterpene for its potential carcinogenic properties. A natural constituent of citrus oils, it also is present in oils from mint, caraway, thyme, cardamom, and coriander. D-limonene is used commercially as a flavor and fragrance.

Interest in limonene stems from its ability to inhibit chemically induced tumors during experimental conditions and its ability to cause the regression of existing tumors *(297)*. Limonene is known to inhibit skin carcinomas *(298)*, mammary carcinomas in c-Ha-ras protooncogene transgenic rats *(299)*, mammary tumors induced by DMBA *(300)*, liver tumors induced by NNM *(301)*, and lung tumors induced by NNK *(302)*. Thus, the potential benefits may be widespread and not limited to a specific tissue. Much of the anticarcinogenic activity brought about during the initiation phase may be mediated through the induction of the hepatic detoxification enzymes GST and uridine diphosphate-glucuronyltransferase *(303)*. Selected changes in CYP enzymes may also contribute to the observed protection *(304)*.

Interestingly, limonene has been reported to cause a complete regression of mammary carcinomas *(300,305,306)*, one of its most impressive effects. Some of this loss of tumor mass is probably explained by the induction of apoptosis *(307)*. Limonene and other monterpenes are known to suppress 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase activity *(308)*. An inhibition of HMG-CoA can deplete intermediates required for the posttranslational modification of proteins, a process that gives proteins lipophilic anchors that bind to membranes. Consequently, nuclear lamins and ras oncoproteins remain in nascent states, and cells do not proliferate. A depression in posttranslational isoprenylation of growth-controlling small G proteins may explain this antitumorigenic property. Ruch and Sigler *(309)* provided evidence that the response does not relate to ras. More recently, Ren and Gould *(310)* found that perillyl alcohol (discussed later) suppressed small G protein isoprenylation in rat mammary glands. The greatest inhibition of small G protein isoprenylation was with RhoA by type I geranyl protein transferase. Rho family proteins serve as guanine nucleotide-regulated binary switches controlling signaling pathways, which ultimately regulate diverse cellular processes, and have been implicated as critical regulators of oncogenesis. Thus, these results may be particularly important. Increased expression of the mannose-6-phosphate/IGF II receptor and TGF-β-1 are recognized responses in cells exposed to monoterpene *(311)*. Ariazi and Gould *(312)* identified 42 induced and 58 repressed genes that were altered by monoterpenes.

The potential of limonene as a chemopreventive compound has led to heightened evaluation of its hydroxylated derivative, perillyl alcohol (POH), as a more potent agent *(313)*. In preclinical studies, cancers of the prostate, esophagus, colon, blood, skin, pancreas, and liver were suppressed by treatment with POH *(297,314–321)*, although there is one report that it exerted tumor-promoting activity in the NNM-induced rodent liver tumor model *(322)*. Since 2000, an increasing number of phase I and II clinical trials have been reported in which the pharmacokinetics and efficacy of POH toward a variety of malignancies *(323–327)* have been examined.

D-limonene has been found to cause kidney tumors when given to male rats at high doses and is associated with the development of hyaline droplet nephropathy *(328)*. The tumor likely arises because of a minor metabolite, D-limonene-1,2-oxide, impeding the normal process of lysosomal proteinase. Because the protein binding this metabolite does not exist in humans, it is likely the tumor would also not occur. Thus, D-limonene may not pose any special carcinogenic or nephrotoxic risk to humans *(328)*.

8. CONCLUSIONS

Variation in the consumption and utilization of food components spanning the fruit, vegetable, and grain domains may contribute to worldwide disparities in the incidence of cancer. Probably keys to this disparity are a host of phytochemicals including flavonoids, isothiocyanates, lignans, allyl sulfurs, and various terpenes. Research continues to provide rather compelling evidence that these and other selected dietary components are capable of modifying cancer risks and tumor behavior. The precise response probably reflects not only the quantity of the constituent consumed, but also its interaction with multiple major and minor genes, as well as with other dietary components.

Epidemiological, clinical, and laboratory investigations provide rather convincing evidence that dietary habits can modify cancer risk. A rather large number of non-nutritive compounds in foods appear to protect against one or more stages of the cancer process. Scientifically, these nonessential nutrients are reported to modify the carcinogenic process by several mechanisms, including altering carcinogen formation and metabolism, curtailing tumor promotion and progression, and modifying cellular and host defenses. Understanding the molecular events that are modified by nutrients will be a key factor in determining the most effective use of diet as a treatment for the prevention of cancer. Although dietary habits are not the sole determinant of cancer, they do represent a significant point for which intervention is possible. Thus, in general, Western populations should modify their lifestyles to include increased intakes of plant foods, therefore reducing the risk ofcancer. Nonetheless, in some cases, adjustment of dietary practices to conform to generalized dietary goals may not be necessary or even appropriate. Unraveling the myriad of possible interactions between specific dietary components and specific genes involved with the cancer process is fundamental to establishing tailored dietary recommendations. Optimizing nutrition by dietary modification continues to be a plausible noninvasive strategy for altering cancer initiation, promotion, and progression.

A greater appreciation of human genetics will hopefully provide valuable insights into identifying those individuals who might benefit or be placed at risk from exaggerated intakes of selected foods. As the era of nutrition and genomics unfolds, it should

become possible to identify tailored diets that can be used to effectively promote health and minimize disease states, including cancer. As this "genomic revolution" emerges, it is essential that the knowledge gained be used responsibly within an ethical framework.

9. RECOMMENDATIONS

A comprehensive understanding about the precise role that specific foods or their components play in the cancer process is fundamental for improving human health. Carefully controlled and probing investigations that examine individual dietary constituents in the context of the entire diet and the environmental factors to which we are exposed will be key in unraveling the true effect of diet in the cancer process. Future research must be aimed at the identification of the critical site(s) or molecular target(s) in which nutrition intervention beyond basic nutriture is most appropriate. Without this information, it will be impossible to tailor recommendations to meet the specific needs of the individual. Although experimental evidence strongly suggests that several nonessential nutrients are significant modifiers of the cancer process, it remains to be determined if humans will benefit from the intake of one or more of these nutrients when taken in isolation from other components of the food. Although individual supplements of nonessential nutrients may be just as effective, their safety must be thoroughly examined not only for toxicity but their dependence on other components of the diet. Until this information is available, it remains prudent to eat a variety of fruits and vegetables and avoid excesses.

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4 Dietary Supplements and Cancer Risk

Epidemiological Research and Recommendations

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KEY POINTS

- Approximately one-half of all Americans use dietary supplements. Thousands of supplements of multiple combinations of vitamins, minerals, and herbs are available for purchase, but the most commonly used supplements are multivitamins and single supplements of vitamins C and E, and calcium.
- Dietary supplements can provide a large proportion of total micronutrient intake for many consumers.
- Individuals who smoke should not use β-carotene supplements, as they have been shown to increase the risk of lung cancer among smokers.
- Vitamin C supplements may reduce the risk of cancers of the gastrointestinal tract and bladder.
- Vitamin E and selenium supplements may reduce prostate cancer risk.
- Calcium may reduce colon cancer risk.

1. INTRODUCTION

Millions of Americans use dietary supplements *(1)*. Data from national nutrition surveys suggest that approx 50% of American adults use dietary supplements *(2,3)*, with substantially higher use among certain population subgroups such as cancer survivors *(4,5)*. One reason for the high prevalence of use is the 1994 passage of Public Law 103–417, the Dietary Supplement and Health Education Act (DSHEA) *(6)*. This legislation discontinued the premarket safety evaluations for ingredients used in supplements, placed the burden of proof of product safety on the US Food and Drug Administration (FDA) instead of the manufacturers, and permitted limited nutrition support statements without prior approval from the FDA *(6)*. These regulations resulted in an exponential increase in the number and variety of dietary supplements available for over-the-counter purchase, including those that consumers believe may prevent chronic diseases such as cancer *(7,8)*.

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The medical community is conflicted about the efficacy of dietary supplements in relation to cancer prevention. Randomized controlled trials of supplements have yielded some entirely unexpected findings. β-carotene, which was believed to prevent cancer, was found to increase the incidence of lung cancer in two large clinical trials *(9,10)*. Selenium, which was hypothesized to reduce the risk of nonmelanomatous skin cancers among persons with previous basal cell or squamous cell carcinomas, had no effect on basal cell cancers, increased the risk of squamous and total nonmelanoma skin cancers, and reduced the risk of several other cancers *(11)*. Selenium is now being tested in a large clinical trial for the primary prevention of prostate cancer *(12)*. Some observational studies suggest that calcium supplements are associated with an increased risk of prostate cancer *(13,14)*, but others have seen a decreased risk of colon cancer *(15–17)*. These results are juxtaposed with a 2002 report that claimed most Americans do not obtain sufficient vitamins from the diet to prevent chronic disease, including certain cancers, and advised that all adults use a daily multivitamin supplement *(18,19)*. Conversely, the US Preventive Services Task Force concluded that there was insufficient evidence to recommend the use of dietary supplements to prevent cancer *(20)*. These opposing views from the medical community and unexpected results from intervention studies underscore the need for a synthesis of the research literature regarding supplements and cancer prevention.

This chapter describes epidemiological evidence to support or refute associations of dietary supplement use with cancer risk. As an introduction, we provide a definition of dietary supplements and briefly review potential biological mechanisms whereby dietary supplements could prevent cancer. We also present methodological considerations important for understanding epidemiological studies on supplement use and cancer risk. The majority of the chapter is devoted to synthesizing results from studies that have provided data on supplement use and cancer risk. We then discuss issues relevant to this research, with emphasis on problems in assessment of supplement use and potential confounding factors. Finally, we provide information on new studies in this area and give our recommendations about the use of dietary supplements to prevent cancer.

1.1. Definition of Dietary Supplements in the United States

Dietary supplements, as defined by the DSHEA, are vitamins, minerals, herbs, or other botanicals and amino acids that are sold as capsules, gel caps, powders, or liquids and are intended to supplement the diet through increased dietary intake *(6)*. These categories are not mutually exclusive, as many supplements contain mixtures of 10 or more micronutrients, herbs, and other potentially bioactive compounds. Highly fortified food products and items intended as the only component of a meal (e.g., meal-replacement beverages) are not classified as dietary supplements.

1.2. Hypothesized Mechanisms of Effect

There is extensive evidence that high consumption of plant foods is associated with lower risk of human cancers *(21–26)*. A comprehensive review of diet and cancer concluded that the current evidence demonstrates a protective effect of vegetable consumption and, less definitively, fruit consumption against almost all major cancers *(27)*. The mechanisms underlying these associations are complex and likely involve numerous compounds and multiple biochemical pathways *(28)*. Included among potential agents

from plant foods are a variety of vitamins (e.g., vitamins C and E and folate) and minerals (e.g., calcium, selenium) as well as a myriad of bioactive compounds such as carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin) and flavonoids (e.g., quercetin, naringenin). The issue of whether dietary supplements containing micronutrients and bioactive compounds found in plant foods would be effective chemopreventive agents is of considerable public health interest. A particularly important point is that the bioavailability of nutrients from supplements has not been completely characterized; for some nutrients, the bioavailability is increased from supplements, whereas for others it is decreased. For example, the bioavailability of folic acid is greater in supplement form compared to natural food forms (e.g., fruits and vegetables), but the bioavailability of supplemental calcium is dependent on the form (e.g., calcium citrate is better absorbed than calcium carbonate) *(29)*.

Laboratory studies provide evidence for mechanisms through which micronutrients commonly found in dietary supplements could prevent, or promote, cancer. Most attention has been focused on nutrients with antioxidant properties: carotenoids, vitamins C and E, and selenium *(30–34)*. There are many potentially relevant functions for antioxidants, including protection of cell membranes and DNA from oxidative damage, scavenging and reduction of *N*-nitroso compounds, and serving as cofactors or structural components for enzymes whose substrates are reactive nitrogen or reactive oxygen species *(24)*. Vitamin E is a nonspecific chain-breaking antioxidant, whereas vitamin C is a strong intra- and extracellular antioxidant via its electron donor capabilities *(35)*. Selenium is a structural component of the glutathione peroxidases, which comprise both the intra- and extracellular antioxidant defense systems *(35)*.

Micronutrients have preventive properties apart from their antioxidant capabilities. Vitamin A (e.g., retinol) plays a role in the differentiation of normal epithelial cells and the maintenance of intercellular communication through gap junctions, thus repressing the processes leading to abnormal cell replication *(36)*. The retinoic acid receptors, RAR and RXR, regulate gene expression of numerous enzymes and proteins and retinoids upregulate the synthesis of natural killer cells and cytokines involved in the inflammatory response *(37)*. Vitamin C enhances the immune response and connective tissue integrity primarily through its role as a cofactor or cosubstrate for enzymes involved in biosynthesis of collagen, catecholamines, and mixed function oxidases *(24,35)*. Vitamin E has strong antiproliferative effects on cultured human tumor cells, possibly mediated by its influence on important cell signaling pathways including transforming growth factor (TGF)-β, c-Jun, and the mitogen-activated protein kinase signaling pathway *(38)*. Folate may be related to cancer risk because inadequate amounts of the vitamin may increase hypomethylation of DNA, with subsequent loss of the normal controls on gene expression *(39)*. Low folate status may also impair DNA repair capacity, a noted risk factor for human cancers *(40)*. Calcium could reduce colon cancer risk by binding bile acids *(41)* or by regulating colorectal epithelial cell proliferation *(15)*. Evidence is suggestive, but not conclusive, that vitamin D reduces the risk of colon and prostate cancer by its crucial role in maintaining calcium homeostasis but perhaps more importantly by inhibiting epithelial cell proliferation and enhancing cellular differentiation *(42,43)*. Selenium may block the clonal expansion of early malignant cells by modulation of cell cycle proteins and apoptotic proteins, in addition to its antioxidant functions *(44)*. Conversely, iron may increase risk of cancer because

it enhances the growth of transformed cells and acts as a pro-oxidant, thereby increasing carcinogenic DNA changes and general oxidative stress *(45,46)*. Improved understanding of cancer biology will be needed for identifying the cellular and molecular processes that can be affected by vitamin and mineral supplementation.

1.3. Prevalence of Supplement Use in the United States

In the United States, consumption of dietary supplements is widespread and has been increasing over the past 15 yr. Based on the 1988–1992 National Health and Nutrition Examination Survey (NHANES III), 35% of males and 44% of females reported taking a dietary supplement in the month prior to the survey *(2)*. The highest levels of use were seen in females aged 50 yr and older (52–55%), those with greater than high school education (50%), and people living in the west (48%). Supplement use in Caucasians (43%) was considerably greater than in African and Mexican-Americans (30%). More recent findings published after the passage of DSHEA suggest that about one-half of adult Americans use supplements *(3,47)*. Importantly, many consumers take multiple supplements either alone or in combination with prescription or over-the-counter medications *(3)*. For example, in a large cohort study of dietary supplement use and cancer risk in western Washington, 32% of the 76,072 cohort participants reported daily use of five or more supplements over the previous 10 yr *(48)*. However, it is important to note that usage patterns among a group of habitual supplement users may differ from the general population. Dietary supplements comprise a substantial portion of American out-of-pocket medical expenditures, estimated at more than $12 billion per year *(1)*.

1.4. Objectives

This chapter presents human observational and experimental data on the use of dietary supplements and cancer risk. To examine the role of supplement use, we included only studies that presented findings on multivitamins or nutrients from supplements, separate from food. Studies on total intake of nutrients (diet plus supplements) are not presented because it is not possible in such studies to separate effects of other bioactive compounds present in foods from those of the specific micronutrients of interest. Hereafter, we use the term "dietary supplement" to include both vitamin and mineral supplements, and we use the term "multivitamin" to refer to a one-a-day type of multivitamin, which typically also contains minerals.

2. BACKGROUND IMPORTANT IN INTERPRETING PUBLISHED STUDIES

2.1. Micronutrient Intakes From Foods and Supplements Differ Markedly

Dietary supplements can provide a large proportion of total intakes of some micronutrients and therefore, the variability in total micronutrient intake attributable to supplements can overwhelm that from foods. For example, in the Women's Health Initiative (WHI) *(49)*, supplement users obtained 50–70% of their total retinol, vitamin C, and vitamin E from supplements, and the median dose from supplements was generally greater than that from foods (Table 1) *(50)*. More recent data from WHI show that antioxidant supplement use increased over time such that compared to 1993–1994, the odds of using single supplements of vitamins C and E in 1998 were 1.37 and 2.10,

Table 1
Vitamin and Mineral Supplement Use Among 16,747 Participants in the Women's Health Initiative

Nutrient	*Taking supplement containing nutrient (%)*	*Intake from supplements among supplement users (median)*	*Intake from foods (median)*	*Supplement intake as % of total intake among supplement users (mean)*	*Supplement intake as % of total intake among all participants (mean)*
Retinol, μg	43.5	2250	439	53.6	35.2
β-carotene, μg	42.6	4500	3264	37.1	24.3
Vitamin C, mg	53.1	200	95	51.4	33.7
Vitamin E, mg	53.2	30	7	71.3	46.7
Folate, μg	44.0	400	245	41.1	26.9
Calcium, mg	51.5	500	672	30.9	20.3
Iron, mg	37.0	18	13	33.4	21.9
Selenium, μg	32.5	20	89	11.3	7.4

From ref. *50*.

respectively *(51)*. These results were remarkably similar to results among men enrolled in a large chemoprevention trial for the primary prevention of prostate cancer. Of the men enrolled in the trial, 44% reported use of a multivitamin on a regular basis and approx 33% used high-dose single supplements of vitamin C or E. Among supplement users, nutrient intake from supplements contributed to about 50% of total β-carotene and folate intakes and approx 60% of vitamins A, C, and D and 90% of vitamin E intakes (Fig. 1) *(52)*. These data illustrate the point that for many nutrients (such as vitamin E), the dose available from supplements (typically 200–1000 mg) is many times larger than can possibly be obtained from foods (about 8–10 mg) *(53)*. Therefore, in many observational studies of cancer risk, the highest levels of intake of many micronutrients could only be obtained from supplements. It follows that some of the significant findings on vitamin or mineral intake were actually detecting an association between supplement use and cancer risk. However, because many of the earlier published studies do not present findings separately for nutrients from foods vs nutrients from supplements, the studies presented here are likely a subset of those that could address associations of supplements with cancer risk. Studies published within the last 5 yr have more consistently presented results separately for nutrients from food vs supplements.

An additional important point regarding micronutrients from food vs supplements is that the chemical isomers in supplements may differ from those in food. This is particularly important for vitamin E. For example, the principal vitamin E isomer in foods is γ-tocopherol, whereas it is almost exclusively α-tocopherol in supplements. These two forms are purported to have distinct biological functions in relation to cancer risk. For example, α-tocopherol is a potent chain-breaking antioxidant because of its electron donating capabilities, whereas γ-tocopherol may be more effective at trapping reactive nitrogen species. γ-tocopherol, but not α-tocopherol, has anti-inflammatory properties and both forms of the vitamin induce apoptosis *(54)*.

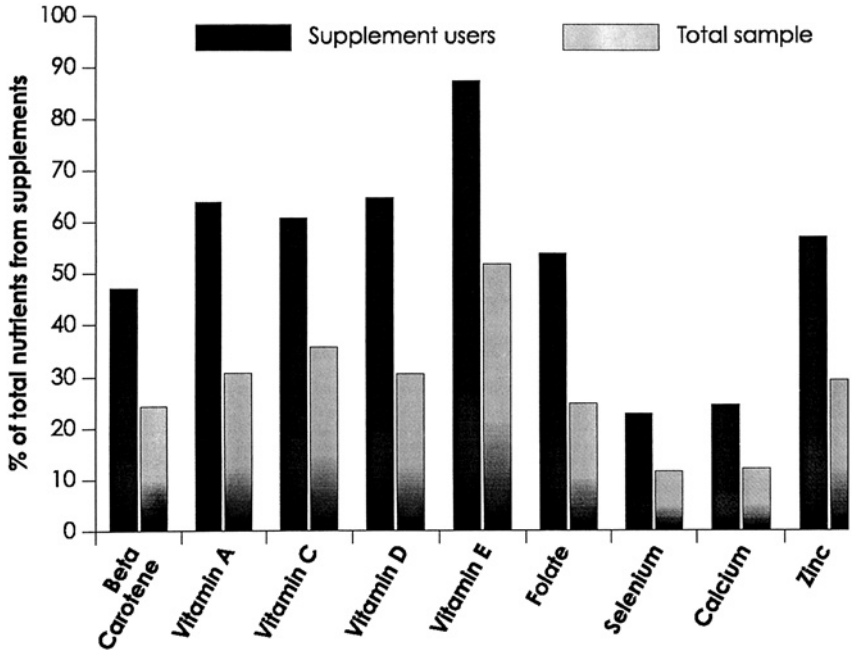


Fig. 1. Nutrient intake among supplement users ($n = 9263$) and complete sample ($n = 15{,}387$) of men enrolled in The Prostate Cancer Prevention Trial *(52)*.

2.2. The Effects of Micronutrients in Multivitamins Cannot Be Isolated

Findings from investigations that measure multivitamin use are a particular analytical challenge because one cannot isolate the potential effect of the micronutrient of interest (e.g., vitamin A) from all the other vitamins and minerals in the supplement. To investigate micronutrients from supplements, study samples must have sufficient numbers of participants using single supplements (e.g., a capsule containing only vitamin A) to separate the effects of these micronutrients from multivitamin use. We suggest that findings on micronutrients that are seldom taken as single supplements (e.g., vitamin A and D, zinc) almost certainly reflect the use of multivitamins and are thus confounded by other constituents. Therefore, we only present observational studies on multivitamins and the following micronutrients: vitamins C and E and calcium. These micronutrients represent the most commonly used single supplements *(55)* and therefore, it is at least plausible that studies of these micronutrients had enough users to isolate their effect from that of multivitamins. There are insufficient epidemiological data addressing herbal supplements and cancer risk to be included in this chapter.

2.3. Issues of Study Design as Related to Research on Vitamin Supplements

Here we briefly describe three study designs used in epidemiological research, with additional comments on their strengths and weaknesses when used to study associations of dietary supplements use with cancer risk.

2.3.1. Randomized Controlled Trials

In randomized controlled trials (RCTs), the investigators allocate the exposure (i.e., the supplement) at random. Participants are then followed over a period of time to

assess the occurrence of a specified disease outcome. Assuming the sample is sufficiently large, the experimental design of an RCT provides a very high degree of assurance about the validity of the results, because both known and unknown characteristics of the intervention and control groups will be identical, thereby eliminating the biases of observational studies, which are described under Subheadings 2.3.2. and 2.3.3. However, RCTs are too costly to conduct multiple trials for different types of cancers, nutrients, doses, combinations, or for long periods of time. The latter point is particularly important because carcinogenesis is a process that takes place over a period of many years; therefore, short-term trials are not likely to yield definitive information. In addition, in many trials the participants are selected to be at high risk for the cancer of interest (e.g., smokers in studies of lung cancer) *(9,10)*, limiting the generalizability of the findings. Finally, prevention trials cannot test an agent with known risk.

2.3.2. Cohort Studies

In cohort studies, the supplement use of a group of disease-free participants is measured. This group is then followed to assess the occurrence of multiple disease outcomes. Cohorts are attractive for studies of supplement use because they can assess the effects of many types, doses, and combinations of supplements, with multiple cancers. Their primary limitation is that the exposure is self-selected, thus investigators must measure and control for factors (such as diet or exercise) likely to confound supplement–cancer associations. In addition, some cohort studies have inadequate statistical power to test associations of supplement use with cancer, because supplement use was relatively rare when these cohorts were established *(55)*, although many cohorts have updated their dietary assessment strategies to include assessment of supplement use *(48,56,57)*.

2.3.3. Case–Control Studies

In case–control studies, participants are selected based on whether they do (case) or do not (control) have cancer. The groups are then compared to see whether supplement use varies by disease status. Selection bias can occur if supplement users are more likely than nonusers to agree to be controls in a study. This bias is probable, because supplement users are more interested in health issues than nonusers *(58)* and, therefore, more likely to participate in a research study. Thus, selection bias may lead to finding a protective effect of a supplement, which may be erroneous. Recall bias can occur if individuals with cancer remember and report their use of supplements differently than controls, which can result in either an overestimation or underestimation of the association of supplement use with cancer risk.

3. REVIEW OF STUDIES ON VITAMIN SUPPLEMENTS AND CANCER RISK

3.1. Methods

Here we summarize the published literature on vitamin supplements and cancer risk, organized by supplement type and by study design. We present results grouped by supplement type because an evaluation of which vitamin supplements are associated with cancer at all sites is important for the development of public health recommendations for the prevention of cancer as a whole. We used the MEDLINE® database of the National Library of Medicine to identify epidemiological studies on dietary supplement

use and cancer risk. We limited our review in several ways. Because few studies presented findings on vitamin supplements before 1985, we searched the database from 1980 forward (up to July 2003). We included a paper only if there were at least 50 cancer endpoints (cases or deaths) in adults. We did not review studies of precancerous conditions (e.g., colorectal adenomas or leukoplakia). We present all data on multivitamins and the other most common single supplements: vitamins C and E and calcium. Additionally, we give data from RCTs of β-carotene and selenium because the unexpected results from these trials have strongly influenced public health recommendations for these nutrients and have motivated the initiation of additional studies *(12)*. As noted earlier, because many other vitamins and minerals (e.g., vitamin A, zinc) are generally only obtained from multivitamin pills, we do not present results for those nutrients.

When available, we present adjusted relative risks (and 95% confidence intervals) for high vs low levels of dietary supplement use (i.e., dose). When relative risks and confidence intervals were not provided, we (a) present relative risks only, (b) calculate relative risks from published tables, or (c) provide a description of findings (e.g., no association).

3.2. Randomized Controlled Trials of β-Carotene

Table 2 gives results from seven RCTs that examined either supplemental β-carotene alone or β-carotene combined with other supplemental nutrients *(9,10,59–66)*. These trials were motivated by observational epidemiology, animal experiments, and mechanistic studies of carcinogenesis. There are strong and consistent findings of protective effects for fruit and vegetable consumption (primary dietary sources of carotenoids), total carotenoid intake, and serum carotenoid concentration on cancer incidence, in particular for cancer of the lung. However, the intervention trials of β-carotene supplements, either alone or combined with other agents, did not support protective effects for incident cancer, cancer mortality, or total mortality. In two studies of persons at high risk of lung cancer, resulting from either smoking or asbestos exposure (The Beta-Carotene and Retinol Efficacy Trial [CARET] and The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study [ATBC]), β-carotene significantly increased lung cancer incidence by 20 and 30%, respectively *(9,10),* whereas a trial conducted in a low-risk population of male physicians and another among female health professionals found no effect of β-carotene on lung cancer incidence *(63)* or total cancer incidence and mortality *(66)*. In CARET, β-carotene also significantly increased lung cancer mortality and cardiovascular disease (CVD) mortality *(10)*. CARET and ATBC continue follow-up with study participants for cancer and mortality endpoints (Gary Goodman, personal communication, 2004) *(64)*. ATBC reported that even after the cessation of the β-carotene intervention, the relative risk for mortality in the β-carotene arm remained elevated for up to 6 yr during the post-trial follow-up. The majority of the excess deaths resulted from cardiovascular events. In terms of cancer incidence in the ATBC follow-up period, there were no statistically significant differences in cancer incidence between β-carotene recipients and nonrecipients at any site, with the exception of a late effect of β-carotene being associated with an 88% increased risk of colorectal cancer *(64)*.

A British trial in 20,536 adults investigated whether a combined supplement of β-carotene and vitamins C and E could reduce the incidence of CVD. Cancer was a secondary endpoint of this trial; there were no statistically significant differences in cancer incidence or mortality at any cancer site between the treatment groups *(65)*. In the single

Table 2
Randomized Controlled Trials of β-Carotene (or Combinations Containing β-Carotene) and Risk of Cancer

Study name (Reference)	*Agent*	*Endpoint(s)*	*Cases*	*Relative risk for supplementation*
Skin Cancer Prevention Study *(59,60)*	50 mg β-carotene	Cancer mortality		
		All sites	82	0.8 (0.5–1.3)[a]
		Incidence		
		Nonmelanoma skin cancer	1,952	1.0 (0.9–1.2)[a]
		Basal cell	651	1.0 (0.9–1.2)[a]
		Squamous cell	132	1.2 (0.9–1.7)[a]
Nutrition Intervention Trials in Linxian, China *(61)*	15 mg β-carotene, 50 μg selenium, and 30mg β-tocopherol	Cancer mortality		
		All sites	792	0.9 (0.8–1.0)
		Esophageal	360	1.0 (0.8–1.2)
		Stomach	331	0.8 (0.6–1.0)
		Cardia	253	0.8 (0.6–1.0)
		Noncardia	78	0.7 (0.5–1.1)
Nutrition Intervention Trials in Linxian, China *(62)*	High-dose (two to three times the Recommended Daily Allowance) multivitamin with minerals and 15 mg β-carotene	Cancer mortality		
		All sites	176	1.0 (0.7–1.3)[a]
		Esophageal	82	0.8 (0.5–1.3)[a]
		Stomach	77	1.2 (0.9–1.9)[a]
		Incidence		
		All sites	448	1.0 (0.9–1.2)[a]
		Esophageal	251	0.9 (0.7–1.2)[a]
		Stomach	177	1.2 (0.9–1.6)[a]
Alpha-Tocopherol, Beta-Carotene Study Intervention period results *(9)*	20 mg β-carotene 50 mg vitamin E	Cancer mortality		
		Lung	564	1.2 (NS)[b]
		Other	552	1.0 (NS)[b]
		Incidence		
		Lung	876	1.2 (1.0–1.4)
		Four cancers	149–250	No associations
Alpha-Tocopherol, Beta-Carotene Study Postintervention Follow-Up *(64)*	N/A	Cancer mortality		
		All sites	1847	
		1993–1996		1.11 (1.03–1.21)[a]
		1996–1999		1.07 (99–1.15)[a]
		Incidence		
		Lung		
		1993–1996	498	1.17 (0.98–1.39)[a]
		1996–1999	539	0.97 (0.82–1.15)[a]
		Colon		
		1993–1996	92	1.06 (0.70–1.60)[a]
		1996–1999	113	1.88 (0.82–1.15)[a]
		All other sites		
		1993–1999	1878	No associations
Physicians' Health Study *(63)*	50 mg β-carotene on alternate days	Cancer mortality		1.0 (0.9–1.2)[a]
		All sites	766	1.0 (0.9–1.1)[a]
		Incidence		
		Nine cancers	50–1047	No associations

Table 2 *(Continued)*

Study name (Reference)	*Agent*	*Endpoint(s)*	*Cases*	*Relative risk for supplementation*
MRC/BHF Heart Protection Study *(65)*	600 mg vitamin C 250 mg vitamin E 20 mg β-carotene	Cancer mortality All sites Incidence All sites Lung Stomach Prostate	704 1617 301 116 290	1.04 (0.90–1.21) 0.98 (0.89–1.08) 1.1 (NS) 1.0 (NS) 0.9 (NS)
Beta-Carotene and Retinol Efficacy Trial or CARET *(10)*	30 mg β-carotene and 25,000 IU vitamin A	Cancer mortality Lung cancer Incidence Lung cancer Prostate cancer Other cancers	254 388 300 730	1.5 (1.1–2.0) 1.3 (1.0–1.6) No associations No associations
Women's Health Study *(66)*	50 mg β-carotene on alternate days	Cancer mortality All sites Cancer Incidence All sites	59 747	1.11 (0.67–1.85) 1.03 (0.89–1.18)

[a]Adjusted relative risk, *see* original studies for details.
[b]Nonsignificant.

trial examining skin cancer, there was no effect of β-carotene on any type of skin cancer or cancer mortality overall *(59,60)*. Two trials in China examined β-carotene combined with other micronutrients. In one trial focused on cancers of the upper digestive tract, supplementation of 15 mg of β-carotene with selenium and vitamin E resulted in a near statistically significant 20% reduction in mortality from stomach cancer *(61)*. In a trial of persons with esophageal dysplasia, supplementation with 15 mg of β-carotene plus high-dose multivitamins with minerals had no effect on total, esophageal, or stomach cancer incidence or mortality *(62)*. The consistent negative or null findings from these clinical trials of supplemental β-carotene are perplexing, given the strong and consistent protective effects of dietary and serum carotenoids found in observational studies *(21–26,67–71)* However, it is clear that high-dose β-carotene supplementation will not reduce the risk of lung cancer among smokers or generally healthy populations.

3.3. Randomized Controlled Trials of Selenium

The Nutritional Prevention of Cancer Trial was designed to test whether selenium supplementation would prevent recurrence of nonmelanoma skin cancers among persons living in parts of the United States where soil selenium levels are low *(72)*. Compared to those in the placebo group, there was a 25% reduction in total cancer incidence and a 40% reduction in cancer mortality. The selenium supplementation reduced prostate and colon cancers by about half, but it statistically significantly increased squamous cell and total nonmelanoma skin cancer by 25 and 17%, respectively, compared to placebo *(11,72,73)*. These findings have generated considerable clinical and scientific interest in selenium's cancer preventive properties *(74,75)*. Selenium, together with vitamin E, is currently being tested in a large, randomized trial for the primary prevention of prostate cancer *(12)*.

3.4. Multivitamins

Multivitamins are the most commonly used dietary supplement. Figure 2 shows results of 28 studies that reported associations of multivitamin use with cancer incidence or mortality *(56,57,62,76–99)*. Only two studies examined all cancers sites, and neither found statistically significant results *(62,76)*. The largest number of studies examined cancers of the upper digestive tract (oral cavity, pharynx, esophagus, and stomach). The single randomized controlled trial found no association with cancers of the upper digestive tract *(62)*. Seven observational studies examined cancers of the oral cavity, esophagus, pharynx, and stomach *(77–79,89,93,95,98,99)*. One found a statistically significant 70% increased risk for oral cancer *(89)*, but among the other studies there were no significant associations or consistent trends. The four studies of breast cancer were inconsistent: two cohort studies found a relative risk near the null value of 1.0 *(80,92)*, one case–control study found a statistically significant 30% increased risk *(81),* whereas another case–control study reported a nonsignificant 27% reduced risk *(96)*. Five out of six studies of colon cancer found reduced risks *(57,82,83,90,91)*, although only two findings of 50 and 75% reduced risks were statistically significant *(83,90)*. Both studies of multivitamins and cervical cancer found statistically significant 40 and 60% reduced risks, respectively *(84,85)*. One study of bladder cancer found a statistically significant 39% reduced risk *(86)*, whereas another reported no association *(94)*. A study of prostate cancer reported no association of multivitamins with disease risk *(97)*. The single study of non-Hodgkin's lymphoma found a doubling in risk for women who used multivitamins over a 10-yr period but no association for men *(56)*.

Studies of multivitamins do not support a protective effect of multivitamins for cancers of the upper digestive tract (oral, pharyngeal, esophageal, and stomach), breast, or prostate. There is limited evidence for a protective effect for cancers of the colon, bladder, and cervix and an increased risk of non-Hodgkin's lymphoma in women.

3.5. Vitamin C

Figure 3 gives results of the 37 studies that reported associations of supplemental vitamin C with cancer incidence or mortality *(56,61,76–80,83–87,89,92–98,100–116)*. Three studies examined all cancer sites combined, and none found either large or statistically significant associations *(61,76,100)*. Several studies examined cancers of the upper digestive tract. The single RCT found no association with cancers of the upper digestive tract *(61)*. Of the seven observational studies, three found statistically significant protective associations: a 50% reduced risk of pharyngeal *(78),* a 30% reduced risk of oral plus pharyngeal *(79)*, and one found a halving in risk, but the results were statistically significant only among the White study participants *(98)*. Two studies found a nonsignificant 25% reduced risk for esophageal cancers *(89,95)*. The studies of stomach cancer found consistent inverse associations with vitamin C, but the confidence intervals around the point estimates all included the null value of 1.0 *(93,95,101)*. The studies of breast cancer found no statistically significant associations or consistent trends *(80,92,96,100,102–104)*. Of the three studies that examined lung cancer, one found a statistically significant 60% reduced risk for men and a nonsignificant 40% increased risk for women *(105)*, whereas results of other studies were inconsistent and none were statistically significant *(100,106)*. Of the studies that examined colon cancer, one found a statistically significant 40% reduced risk *(83)*, two found nonstatistically

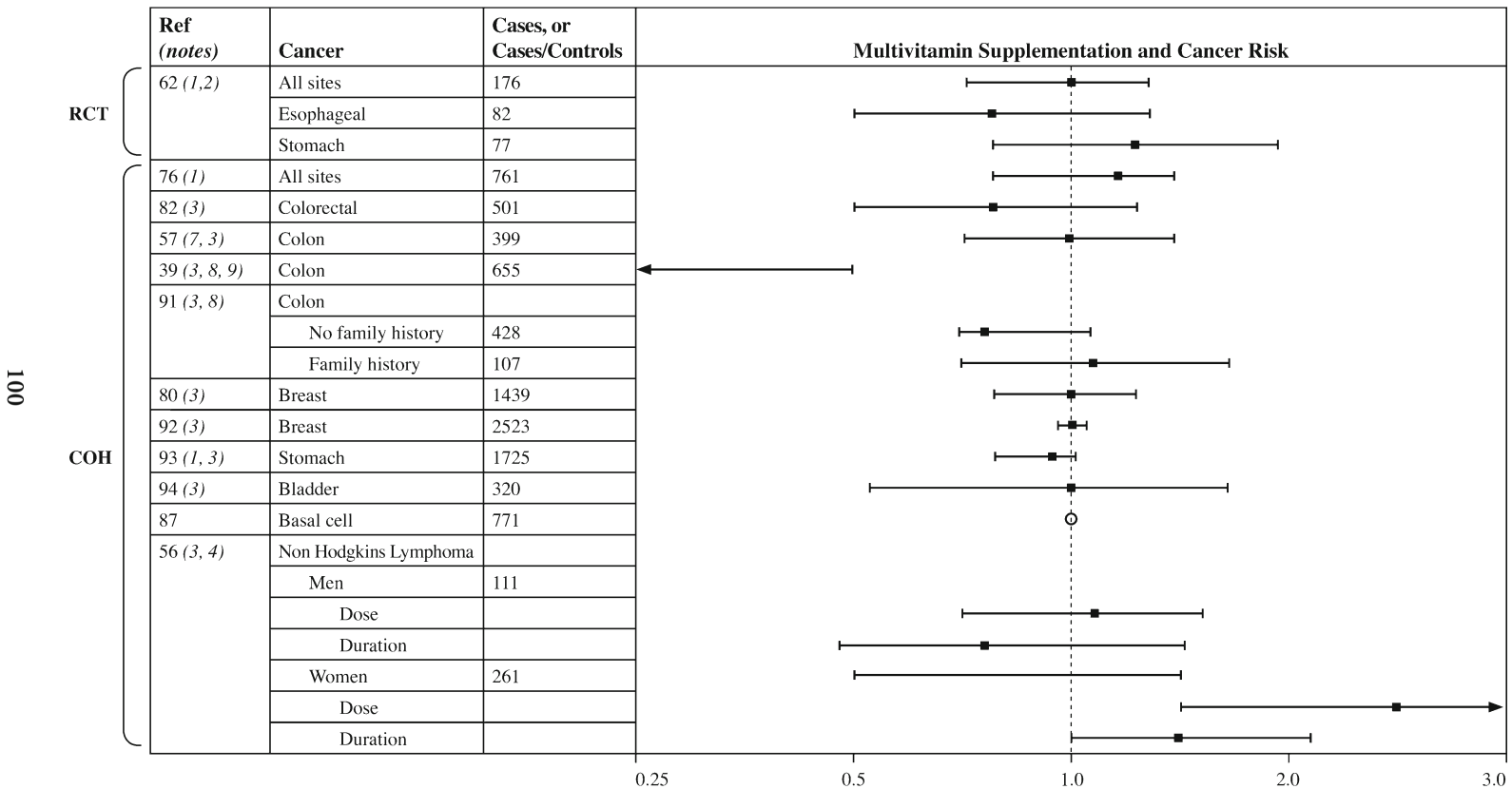
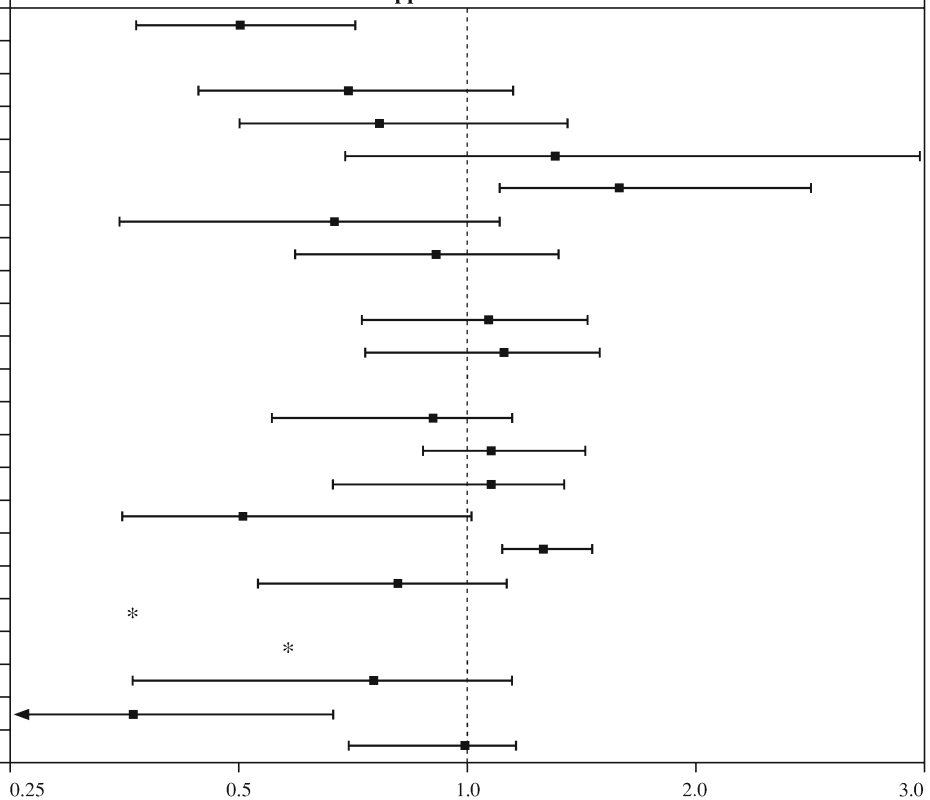


Fig. 2. Associations of multivitamin use and cancer risk.

C/C

Ref *(notes)*	Cancer	Cases, or Cases/Controls
83 *(4,5)*	Colon	444/427
98 *(5)*	Esophageal	
	White	114/681
	Black	219/554
89 *(5)*	Esophageal	133/264
	Oral	290/576
77 *(5)*	Esophageal	174/750
78 *(5)*	Pharyngeal	166/547
95 *(5)*	Esophageal	
	Adenocarcinoma	282/687
	Squamous cell	206/687
	Stomach	
	Cardia	255/687
	Noncardia	352/687
79 *(5)*	Oral and pharyngeal	1103/1262
99 *(5)*	Oral and pharyngeal	133/212
81 *(5,6)*	Breast	1486/1336
96 *(5)*	Breast	861/790
84 *(5)*	*In situ* cervical	229/502
85 *(5)*	Invasive cervical	271/502
88 *(5)*	Melanoma	234/248
86 *(5)*	Bladder	262/405
97 *(5)*	Prostate	697/666



[1] Number of deaths
[2] High dose multivitamins with minerals, including 15 mg beta-carotene
[3] Adjusted relative risk
[4] Average dose per year over a 10-year reference period
[5] Adjusted odds ratio
[6] Vitamins ABCDE and minerals. Other mixtures included ABCD with or without minerals, and ABCDE without minerals, and ABCDE with selenium only. None of the other mixtures had statistically significant relative risks.
[7] Males only
[8] Females only
[9] Point estimate is below the scale shown.

Fig. 2. (*Continued*)

significant reduced risks *(100,107)*, and one found a nonstatistically significant reduced risk for women only *(108)*. Both studies of cervical cancer found statistically significantly reduced risks—50% for *in situ (84)* and 30% for invasive *(85)*. Results from the six studies that examined bladder cancer were inconsistent. One study found a statistically significant 40% reduced risk for men only *(100)*. Of the remaining studies, one found a statistically significant 60% reduction *(86)*, two found a nonsignificant 30–50% reduced risk *(94,109)*, whereas another found a 50% reduced risk in women and a 20% increased risk in men, both of which were nonstatistically significant *(110)*. Only one cohort study reported a nonsignificant 25% increased risk for bladder cancer mortality associated with use of vitamin C supplements *(113)*. The single study of ovarian cancer reported a statistically significant halving in risk associated with use of supplemental vitamin C *(115)*. Studies of basal cell carcinoma, prostate cancer, and non-Hodgkin's lymphoma did not find any statistically significant associations with vitamin C use *(56,87,97,114,116)*.

Studies of vitamin C do not support a protective effect for total, breast, prostate, or lung cancers. There is modest evidence for a protective effect for cancers of the upper digestive tract, cervix, ovary, bladder, and colon.

3.6. Vitamin E

Figure 4 shows results for the 34 studies that have reported associations of supplemental vitamin E with cancer incidence or mortality *(56,61,76,79,80,83–87,89,92,93, 95–97,100–104,107,108,111–115,117–121)*. Only three studies report associations with all cancer sites combined; two found nonstatistically significant 20% *(61,100)* reductions in risk, and one found a nonstatistically significant 60% reduction in risk *(76)*. One of these studies was an RCT that combined vitamin E with β-carotene and selenium, thus its result cannot be attributed to vitamin E alone *(61)*. None of the seven studies of breast cancer found statistically significant associations or consistent trends *(80,92,96,100,102–104)*. Of the three studies on lung cancer, only the study among nonsmokers found a significant 50% reduced risk for both men and women *(118)*. Importantly, the ATBC Study, which was a randomized trial that included one study arm designed specifically to test whether vitamin E could prevent lung cancer in smokers, found no effect *(9)*. However, an unexpected finding in this trial was a statistically significant 30% reduced risk for prostate cancer in the vitamin E arm of the trial, as noted earlier *(117)*. Subsequently, a cohort study reported a modest increased risk of late-stage prostate cancer with vitamin E *(121)*, but another cohort study and a case–control study found nonstatistically significant 24 and 30% reduced risks *(97,116)*. The selenium and

Fig. 2. Results of studies of multivitamin supplementation and cancer risk: relative risks and odds ratios with 95% confidence intervals, in which a point estimate of less than 1.0 indicates that multivitamins reduce the risk of cancer and a confidence interval that does not include 1.0 indicates that the finding was statistically significant ($p < 0.05$). A "ↄ" indicates that there was no statistically significant association, and the relative risk or odds ratio was not presented in the original manuscript. A "*" indicates that there was a statistically significant association, but the confidence intervals were not given in the original manuscript. An arrow indicates that the confidence interval exceeds the scale. RCT, randomized controlled trial; COH, cohort study; C/C, case–control study.

	Ref *(notes)*	Cancer	Cases, or Cases/Controls
RCT	62 *(1,2)*	All sites	792
		Esophagus	360
		Stomach	331
		Cardia	253
		Noncardia	78
COH	100 *(3)*	Men:	
		All sites	645
		Lung	94
		Colon	97
		Prostate	208
		Bladder	71
		Women:	
		All sites	690
		Lung	70
		Colon	105
		Breast	219
	76 *(1,3)*	All sites	761
	108 *(3)*	Men:	
		Colorectal	58
		Women:	
		Colorectal	68
	107	Colon	212
	112 *(1,3)*	Colon	4404
	93 *(1,3)*	Stomach	1725
	80 *(3)*	Breast	1439
	102 *(3,4)*	Breast	519
	103 *(3)*	Breast	879
	92 *(3)*	Breast	2523
	116 *(3)*	Prostate	642
	87	Basal cell	771

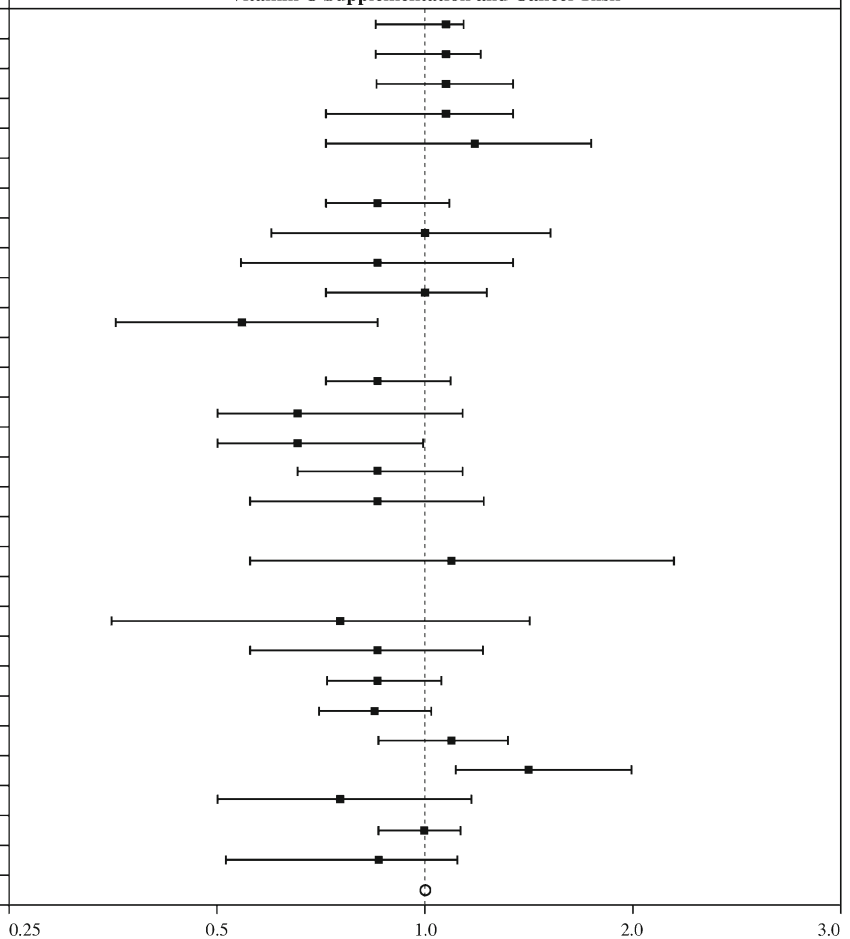


Fig. 3. Associations of vitamin C with cancer risk.

	Ref *(notes)*	Cancer	Cases, or Cases/Controls
COH	114 *(3)*	Basal cell	5392
	113 *(1,3,7)*	Bladder	1289
	94 *(3, 7)*	Bladder	320
	56 *(3, 6, 8)*	Non-Hodgkins Lymphoma	
		Men	111
		Dose	
		Duration	
		Women	261
		Dose	
		Duration	
C/C	83 *(5,6)*	Colon	444/427
	98 *(5, 8)*	Esophageal	
		White	114/681
		Black	219/557
	89 *(5)*	Men:	
		Esophageal	133/264
		Oral	290/576
	77 *(5)*	Men:	
		Esophageal	174/750
	101 *(5)*	Stomach	338/679
	78 *(5)*	Pharyngeal	166/547
	79 *(5)*	Oral and pharyngeal	1103/1262
	95 *(5)*	Esophageal	
		Adenocarcinoma	282/687
		Squamous cell	206/687
		Stomach	
		Cardia	255/687
		Noncardia	252/687
	104 *(5)*	Breast	297/311
	96 *(5)*	Breast	861/790
	84 *(5)*	*In situ* cervical	229/502

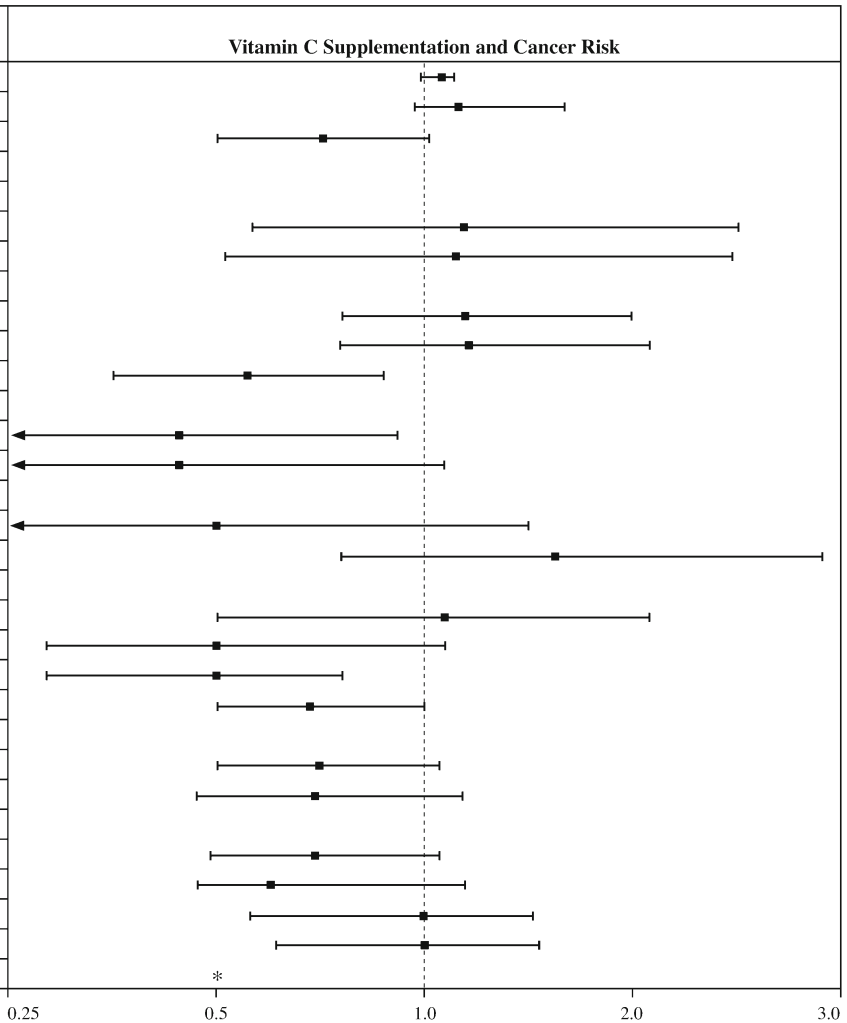
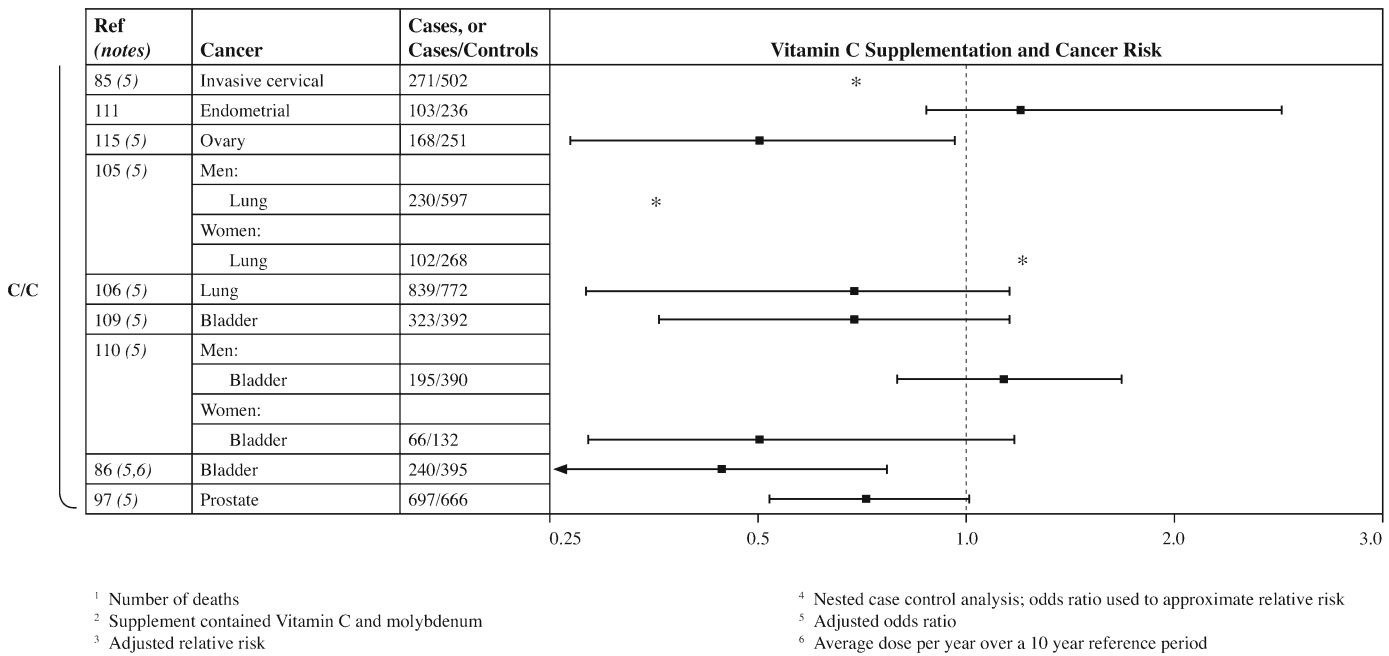


Fig. 3. (*Continued*)



[1] Number of deaths
[2] Supplement contained Vitamin C and molybdenum
[3] Adjusted relative risk
[4] Nested case control analysis; odds ratio used to approximate relative risk
[5] Adjusted odds ratio
[6] Average dose per year over a 10 year reference period

Fig. 3. Results of studies of vitamin C supplementation and cancer risk: relative risks and odds ratios with 95% confidence intervals, in which a point estimate of less than 1.0 indicates that vitamin C reduces the risk of cancer and a confidence interval that does not include 1.0 indicates that the finding was statistically significant ($p<0.05$). A "○" " indicates that there was no statistically significant association, and the relative risk or odds ratio was not presented in the original manuscript. A "*" indicates that there was a statistically significant association, but the confidence intervals were not given in the original manuscript. First column gives types of study. RCT, randomized controlled trial; COH, cohort study; C/C, case–control study.

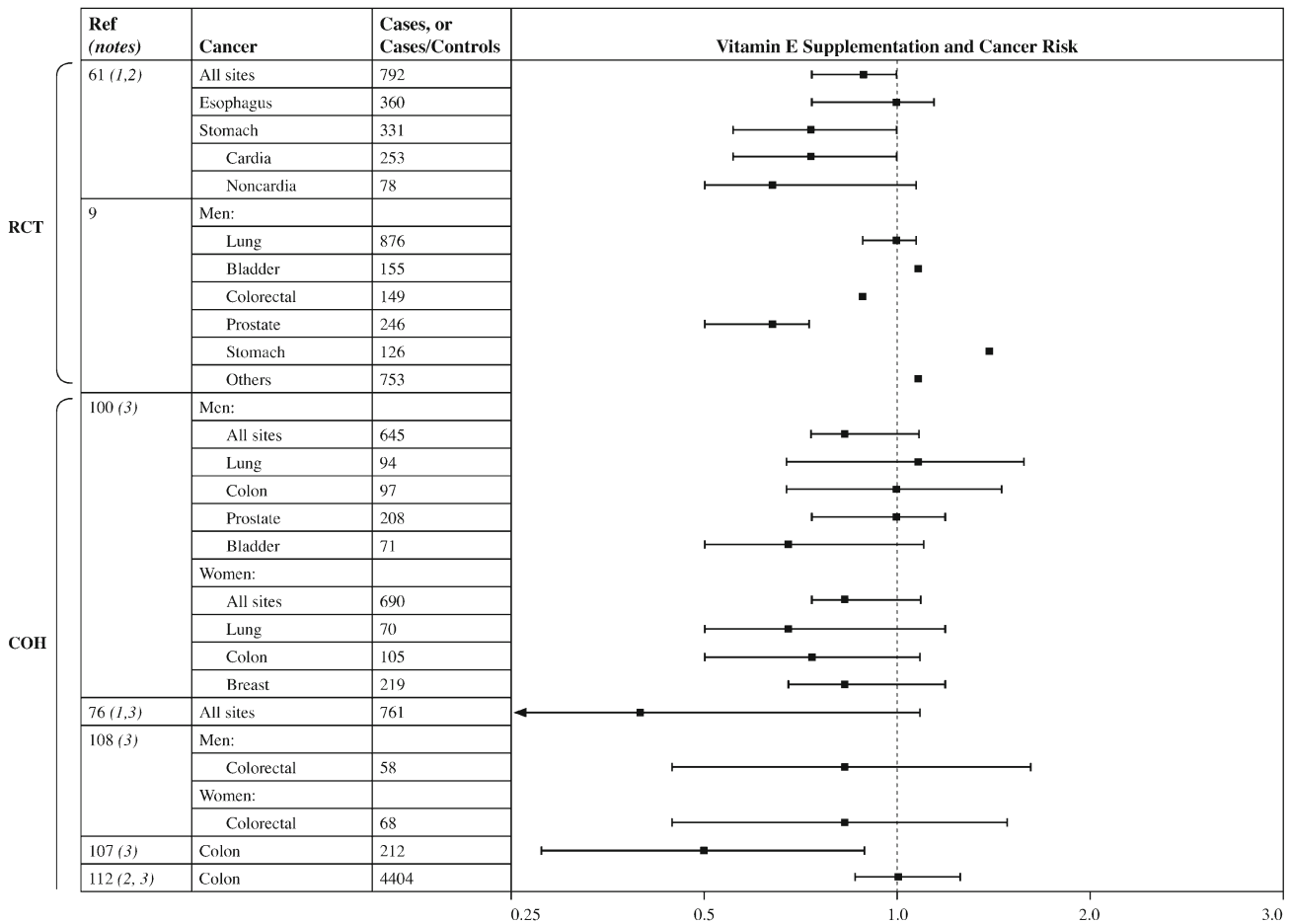


Fig. 4. Associations of vitamin E with cancer risk.

COH

Ref *(notes)*	Cancer	Cases, or Cases/Controls
93 *(2, 3)*	Stomach	1725
57	Men	266
	Colon	
	Dose	
	Duration	
	Women	448
	Colon	
	Dose	
	Duration	
102 *(3,4)*	Breast	519
80 *(3)*	Breast	1439
103 *(3)*	Breast	879
92 *(3)*	Breast	2523
116 *(3)*	Prostate	642
121 *(3)*	Prostate (early stage)	1896
	Prostate (late stage)	522
113 *(2, 3)*	Bladder	1289
87	Basal cell	771
114 *(3)*	Skin (Basal cell)	5292
56 *(3, 6)*	Non-Hodgkins Lymphoma	
	Men	111
	Dose	
	Duration	
	Women	261
	Dose	
	Duration	

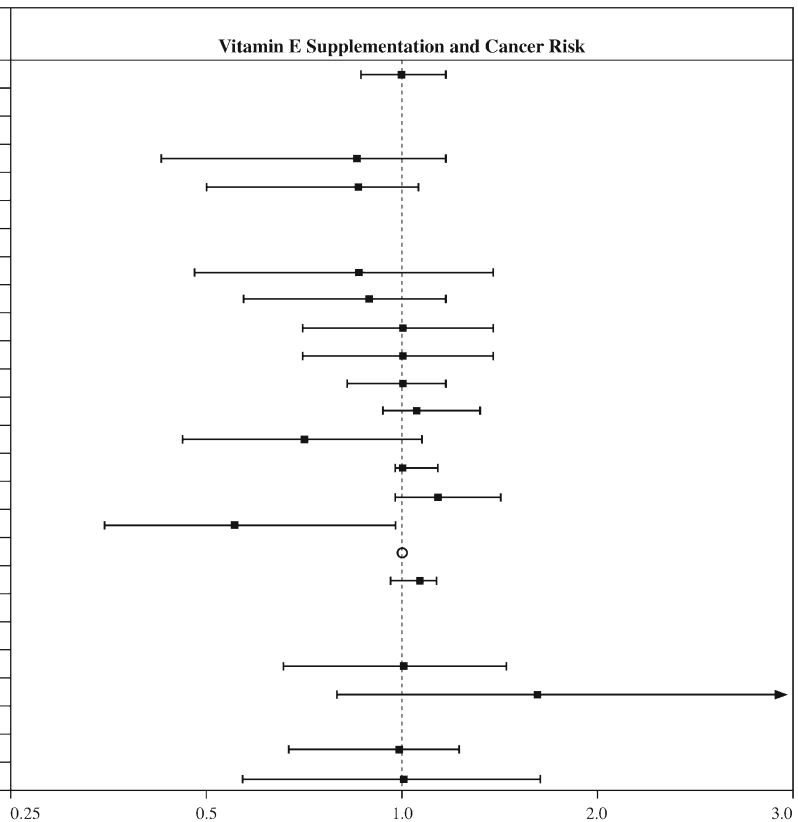
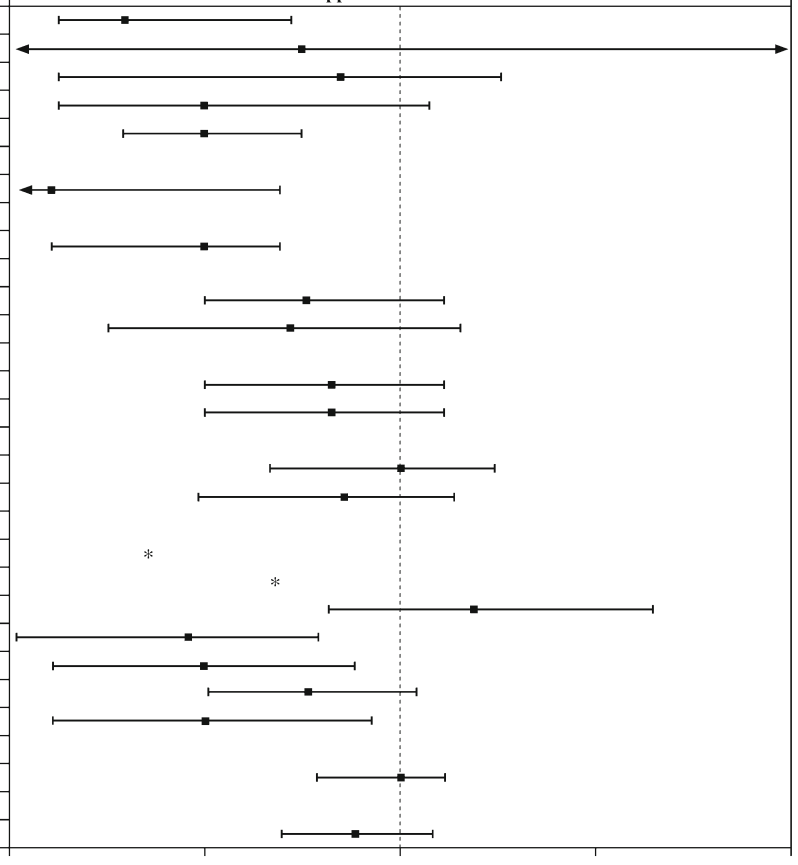


Fig. 4. (*Continued*)

C/C

Ref *(notes)*	Cancer	Cases, or Cases/Controls
83 *(5,6)*	Colon	444/427
89 *(5)*	Esophageal	133/264
	Oral	290/576
101 *(5)*	Stomach	338/679
79 *(5)*	Oral and pharyngeal	1,103/1,262
119 *(5)*	Blacks:	
	Oral and pharyngeal	194/979
	Whites:	
	Oral and pharyngeal	194/203
95 *(5)*	Esophageal	
	Adenocarcinoma	282/687
	Squamous cell	206/687
	Stomach	
	Cardia	255/687
	Noncardia	352/687
104 *(5)*	Premenopausal women:	
	Breast	297/311
96 *(5)*	Breast	861/790
84 *(5)*	White women:	
	In situ cervical	229/502
85 *(5)*	Invasive cervical	271/502
111 *(5)*	Endometrial	103/236
115 *(5)*	Ovary	168/251
118 *(5)*	Nonsmoking lung	413/413
97 *(5)*	Prostate	697/666
86 *(5,6)*	Bladder	240/395
120 *(5)*	Men:	
	Colon	1099/1290
	Women:	
	Colon	894/1120



[1] Vitamin supplement contained β-carotene, selenium, and α-tocopherol
[2] Number of deaths
[3] Adjusted relative risk
[4] Nested case-control analysis; odds ration used to approximate relative risk
[5] Adjusted odds ratio
[6] Average dose per year over 10-year reference period

Fig. 4.

Vitamin E Chemoprevention Trial (SELECT) is currently in progress to test whether a combination of vitamin E and selenium is effective for the primary prevention of prostate cancer *(12)*.

Twelve studies examined associations of vitamin E with upper digestive tract cancers. Of the two RCTs, the trial examining the vitamin E, selenium, and β-carotene combined found a near statistically significant 20% reduction in stomach cancer *(61)*, whereas the other trial of vitamin E alone found a nonstatistically significant 30% increased risk *(9)*. Of the three studies that examined oral cancer alone, or oral plus pharyngeal cancer, two found statistically significant protective effects ranging from a 50 to 70% reduced risk *(79,119)*. The remaining studies of upper digestive tract cancers found either no associations *(93)* or nonstatistically significant protective associations *(89,95,101)*. Of the eight studies that examined colon cancer, two observational studies found statistically significant protective associations of 50 and 60% reduced risks *(16,83),* whereas the others found only modest, nonstatistically significant inverse associations. For example, an RCT found a nonsignificant 20% reduced risk *(9)*, and three of four observational studies found small reduced risks in women but not men *(100,108,120)*, whereas one reported nonsignificant reduced risks of similar magnitude for both men and women *(57)*. Vitamin E supplements significantly reduced ovarian cancer risk by 57% and cervical cancer risk by 40 to 60% in the few studies of gynecological cancers *(84,115)*. Two studies of bladder cancer found 50 to 60% reduced risks associated with vitamin E supplementation *(86,113)*.

Studies of vitamin E do not support an association with breast or smoking-related lung cancers. There is a single study of a protective effect for prostate cancer in an RCT, but the inconsistent results from observational studies suggest that waiting for the results from SELECT will be important before public health recommendations can be formulated regarding vitamin E and prostate cancer prevention. There is modest evidence for protective associations of vitamin E with cancers of the upper digestive tract, colon, bladder, and the female reproductive tract.

3.7. Calcium

Figure 5 gives results of 15 studies that have reported associations of calcium supplementation with cancer incidence *(13,16,17,83,95,111,122–129)*. Of the studies that examined colon cancer, two found a statistically significant reduced risk *(122,127)*,

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Fig. 4. Results of studies of vitamin E (AT) supplementation and cancer risk: relative risks and odds ratios with 95% confidence intervals, in which a point estimate of less than 1.0 indicates that vitamin E reduces the risk of cancer and a confidence interval that does not include 1.0 indicates that the finding was statistically significant ($p < 0.05$). A "○" "indicates that there was no statistically significant association, and the relative risk or odds ratio was not presented in the original manuscript. A "■" indicates that there was no statistically significant association, and only the relative risk or odds ratio is presented in the original manuscript, but not the confidence intervals. A "*" indicates that there was a statistically significant association, but the confidence intervals were not given in the original manuscript. First column gives types of study. RCT, randomized controlled trial; COH, cohort study; C/C, case–control study.

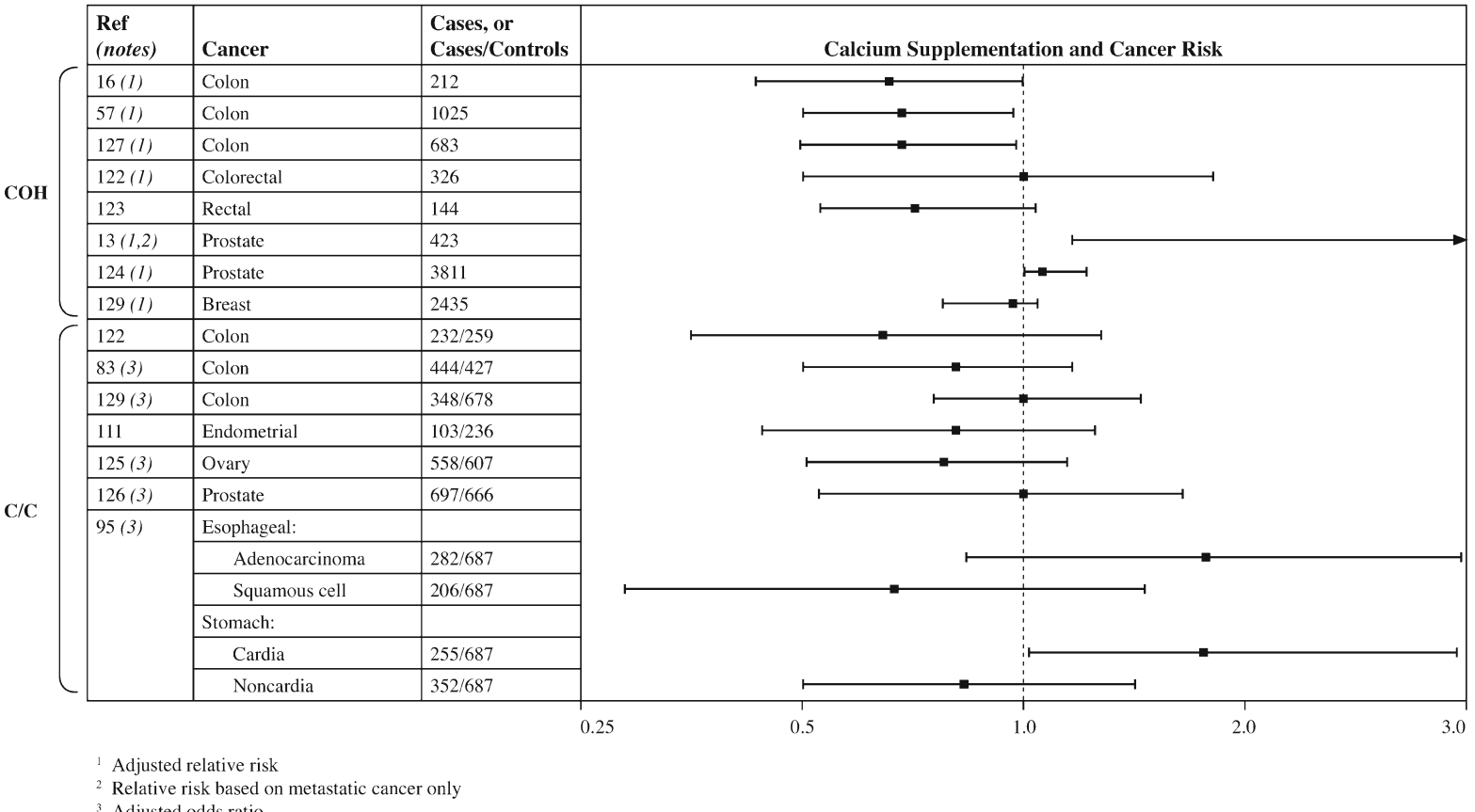

Fig. 5. Associations of calcium with cancer risk.

three found nonstatistically significant 20 and 30% reduced risks *(16,17,83)*, and two reported no association *(122,128)*. The single report for rectal cancer found a near statistically significant 24% reduced risk *(123)*. The data on prostate cancer are inconsistent; one cohort study found a large (approx 300%) and statistically significant increased risk *(13)* and another reported a modest 60% increased risk associated with calcium intake *(124)*. However, the exceptionally wide confidence intervals in one study *(13)* and the small number of cases in the highest quintile of calcium intake in the other *(124)* suggest that we view these findings with caution. Notably, a case–control study reported no association of supplemental calcium with prostate cancer risk *(126)*, but a later publication from that study suggested a modest association of use of calcium supplement with increased risk for metastatic prostate cancer (126). There were nonstatistically significant associations of calcium supplementation with cancers of the endometrium *(111)* and ovary *(125)* and inconsistent associations with cancers of the upper digestive tract *(95)*.

Studies of calcium supplementation and cancer are limited. There is modest evidence for a protective association of calcium supplementation with colon cancer risk.

4. DISCUSSION

This chapter provides modest support for an association of dietary supplement use with cancer risk. The strongest findings are from the RCTs that indicate β-carotene can increase incidence of cancer in smokers, α-tocopherol can reduce risk of prostate cancer, and selenium may influence a broad range of cancers. No supplements appear to be definitively related to breast cancer. Cancers of the gastrointestinal tract appear to be inversely associated with all the supplements examined here, with perhaps the most consistent associations seen between multivitamins, vitamin E, calcium, and colon cancer, and vitamin C with both upper and lower gastrointestinal cancers.

There are a number of methodological problems in much of the epidemiological research on dietary supplement use. These limitations are important to consider before drawing conclusions from this chapter. Here we discuss three important issues: (a) measurement error in assessment of supplement use, (b) importance of a time-integrated measure, and (c) supplement use as a marker for cancer-related behavior.

4.1. Measurement Error in Assessment of Supplement Use

Considerable controversy exists regarding the validity of dietary data obtained in epidemiological studies. However, less is known about instruments used to assess

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Fig. 5. Results of studies of calcium supplementation and cancer risk: relative risks and odds ratios with 95% confidence intervals, in which a point estimate of less than 1.0 indicates that calcium was associated with a reduced risk of cancer and a confidence interval that does not include 1.0 indicates that the finding was statistically significant ($p < 0.05$). A "*" indicates that there was a statistically significant association, but the confidence intervals were not given in the original manuscript. An arrow indicates that the point estimate and confidence interval exceed the scale. COH, cohort study; C/C, case–control study. There were no published randomized controlled trials of the calcium supplementation and cancer incidence of mortality.

supplement use. Epidemiological studies typically use personal interviews or self-administered questionnaires to obtain information on three to five general classes of multivitamins and on single supplements, the dose of single supplements, and sometimes frequency and/or duration of use. We have dedicated a considerable effort toward validating dietary supplement collection instruments.

In 1998, we published results from a validation study comparing supplement data collected in a telephone interview and from a brief self-administered questionnaire with data derived from a detailed in-person interview and transcription of the labels of supplement bottles (i.e., a gold standard) among adult supplement users in Washington ($n = 104$). Correlation coefficients comparing average daily supplemental vitamin and mineral intake from the interview or questionnaire to the gold standard ranged from 0.8 for vitamin C to 0.1 for iron *(130)*. These results suggest that commonly used epidemiological methods of assessing supplement use may incorporate significant amounts of error in estimates of some nutrients. The effect of this type of nondifferential measurement error is to attenuate measures of association, which could obscure many significant associations of supplement use with cancer.

More recently, we completed a validity study of a very extensive and detailed 24-page instrument of dietary supplement use in comparison with an array of nutritional biomarkers. In a sample of 220 adults aged 50 to 74 yr, there were modest correlations of self-reported use of supplemental intake with serum concentrations of vitamin C ($r = 0.29$) and β-carotene ($r = 0.31$) and a very good correlation with serum vitamin E ($r = 0.69$) *(131)*. The high correlation with serum vitamin E might suggest that this self-administered supplement questionnaire was quite accurate, with the lower correlation for the other biomarkers resulting from other influences on those serum measures.

4.2. Importance of a Time-Integrated Measure of Supplement Use

Investigators studying diet and chronic diseases usually wish to measure an individual's long-term nutrient intake because the induction and latent periods for these diseases are long *(21,22)*. However, many studies only asked participants about their current use of vitamin and mineral supplements or only obtained information about supplement use at one point in time. Potential sources of variability in supplement use over time include changes in (a) the type of multivitamin used, (b) number of years the supplement was taken, (c) formulations of multivitamins or dose of single supplements, and (d) frequency of taking supplements.

We conducted a mail survey to investigate the relationship between current and long-term (10-yr) supplement use ($n = 325$ adults) *(132)*. Estimates of current daily intakes for supplemental micronutrients were roughly twice that of average daily intake over the previous 10 yr. Correlations between current intake and long-term intake from supplements alone were 0.77, 0.75, and 0.65 for vitamins C, E, and calcium, respectively *(132)*. This type of measurement error may also have contributed to many of the null associations in this chapter.

4.3. Supplement Use as a Marker for Cancer-Related Behavior

Observational studies on supplement use can be seriously compromised by confounding information because supplement use is strongly related to other factors that affect cancer risk. Supplement users are more likely than nonusers to be female, Caucasian, better

educated, affluent, nonsmokers, light drinkers, and to consume diets lower in fat and higher in fiber and some micronutrients *(133–137)*. However, one set of potential confounding variables only recently explored are those specific to cancer risk: screening, use of potentially chemopreventive agents, and diet-related attitudes and behavior.

Demographical and health-related characteristics of high-dose supplement users were assessed as part of a cohort study of dietary supplement users and cancer risk in western Washington. Among women, those who had a mammogram in the previous 2 yr were 60% more likely to be users of calcium, 50% more likely to use multivitamins, and 20 and 40% more likely to use vitamins C and E, respectively, than women who did not have a mammogram. Among men, those who had a prostate-specific antigen (PSA) test within the previous 2 yr were about 1.5 times more likely to be users of vitamin E and 40% more likely to be users of multivitamins and vitamin C than men who did not get a PSA test. For both men and women, there was a strong positive association between having a sigmoidoscopy, using nonsteroidal anti-inflammatory drugs and using multivitamins and single supplements. High-dose supplement users were statistically significantly more likely to be of normal weight, be nonsmokers, exercise regularly, and eat five or more servings fruits and vegetables per day *(48)*. These results are similar to our results from a random-digit-dial survey to monitor cancer risk behavior in adults in Washington state (n = 1449) *(58)*.

These relationships could confound studies of supplement use and cancer risk in complex ways. For example, male supplement users were more likely to have had a PSA test, which is associated with increased diagnosis of prostate cancer. Thus, supplement users could appear to have a higher incidence of prostate cancer. However, if early diagnosis of prostate cancer by PSA reduces mortality, supplement users could appear to have lower prostate cancer mortality. Health beliefs influence cancer risk through behavior such as diet and exercise. For example, in a previous prospective study, we found that belief in a connection between diet and cancer was a statistically significant predictor of changes to more healthful diets over time *(138)*. Cohort studies showed that the increasing healthfulness of supplement users' diets and other health practices over time could result in a spurious positive association between supplement use and chronic disease.

In theory, control in analyses for demographics and health-related behavior adjusts for these confounding factors. However, absence of residual confounding cannot be assured, especially if important confounding factors are unknown, not assessed, or not included in the analyses.

4.4. Future Research

Despite the large number of studies reviewed in this report, the published studies to date on dietary supplements and cancer risk are far from definitive. Research on dietary supplements must continue before public health recommendations can be formulated. The National Institutes of Health appears to be committed to research on dietary supplements. The 1994 DSHEA legislation mandated the creation of the Office of Dietary Supplements (ODS) *(6)*. The ODS supports research, sponsors workshops and consensus conferences, and disseminates information about dietary supplements to researchers, clinicians, and consumers. One goal of some of the ODS-funded research is to establish a network of Dietary Supplement Research Centers *(139)*. The ODS can be accessed at http://dietary-supplements.info.nih.gov/. The website contains links to

several of the dietary supplement consensus conferences, which have occurred over the last several years.

Several large projects funded by the National Institutes of Health are in progress. Specifically regarding supplements, the WHI is testing other outcomes such as fracture risk and whether a combined dose of calcium and vitamin D will reduce the incidence of colorectal cancer in postmenopausal women *(49)*; results are expected in 2005. The SELECT Study is underway, but results are not expected until 2014 *(12)*. The Physicians' Health Study II is a randomized trial of β-carotene, vitamin E, vitamin C, and multivitamins among healthy, male physicians to test whether these supplements will reduce the incidence of total and prostate cancers, as well as CVD and eye diseases *(140)*. Results from a French randomized trial to test whether antioxidant supplements can reduce cancer incidence and cancer mortality are expected soon *(141,142)*. A cohort of more than 77,000 residents of western Washington called VITamins And Lifestyle Study (VITAL) is examining associations of dietary supplement use with cancer incidence. Recruitment was completed in 2002, and reports on specific cancers are expected within the next 5 yr *(48)*. These and other investigations are expected to yield important data on specific dietary supplements in relation to cancer risk that will be useful to both scientists and clinicians.

5. RECOMMENDATIONS

Increasing numbers of Americans are using dietary supplements. Health professionals are confronted regularly with questions regarding the efficacy of these compounds. Physicians must remain informed about research showing efficacy, harm, or no effect of dietary supplements in relation to cancer prevention.

Most supplement users believe that these compounds improve their health. In a small study on motivations and beliefs of supplement users (n = 104), we found that supplement users believed that multivitamins helped them feel better (41%), vitamin C prevents colds and/or flu (76%), and vitamin E and calcium prevent chronic disease (60–80%) *(52)*. Many participants felt that foods could not supply adequate amounts of certain nutrients, indicating that advice to "eat a balanced diet" would not reduce their motivation to take dietary supplements.

Given the large numbers of Americans taking dietary supplements, we believe that it is important to formulate recommendations regarding their use. We feel the following recommendations regarding dietary supplements and cancer risk are consistent with the literature and are responsible:

- Results of RCTs clearly indicate that cigarette smokers, or other individuals at high risk for lung cancer, should not take β-carotene supplements. Healthy adults will receive no benefit from β-carotene supplementation.
- The evidence regarding selenium and cancer is intriguing and biologically plausible. The formulation of specific recommendations for the public should be made after the results are known from the RCTs currently in progress *(12)*. Another remaining question is that little is known about which forms of the mineral may yield the most benefits: the so-called food forms of selenium in yeast vs inorganic forms of selenium (selenate or selenite, the latter of which is relatively instable) *(143)*. The SELECT trial is testing the organic form of selenium as 200 µg of seleomethionine *(12)*. If an individual chooses supplementation, we recommend that the daily dose not exceed 200 µg, which appears to have been safe in the US intervention trial *(72)*.
- A daily multivitamin and mineral pill is unlikely to be harmful and may be beneficial.

- Vitamin C supplementation may reduce the risk of some cancers, particularly those of the gastrointestinal tract and the bladder. Anecdotal evidence indicates that doses as high as 1 g/d are safe *(144)*. However, pharmacokinetic evidence suggests that there is a ceiling effect of oral vitamin C on plasma levels *(145)*. Therefore, higher doses of C may not offer an increase in benefit.
- Studies to date suggest that vitamin E supplementation may reduce risk of prostate or colon cancer. The toxicity of vitamin E is low, and clinical trials indicate that large doses (200–800 mg/d) do not result in serious side effects in most adults *(146)*. Therefore, doses in this range are acceptable.
- There is evidence that calcium supplements may reduce risk of colon cancer. The results from the WHI are expected to provide important information about calcium in relation to colon cancer risk in postmenopausal women. Given the much stronger evidence that this supplement can prevent osteoporosis *(147)*, use of calcium supplements in the range of 500 to 1000 mg/d may be prudent for many Americans. Additionally, there is modest evidence that folic acid reduces colon cancer risk *(90,91)*. Limitations of the currently published studies are that many of the outcomes are intermediate endpoints (e.g., adenomas) and the fact that few individuals use single supplements of folic acid. Further research is needed in this area.
- There is little epidemiological research on other vitamin (e.g., vitamins A, B_1, B_2, B_6, and B_{12}), mineral (e.g., chromium, copper, magnesium, iron, zinc) and herbal supplements and cancer. Additionally, there is some emerging and compelling data to suggest a preventive role for vitamin D in relation to breast, prostate, and colon cancers *(43,148)*. However, most Americans obtain vitamin D either from milk products or multivitamins. Despite the increasing publications of animal and in vitro studies of the potential role for vitamin D in carcinogenesis, few use single supplements of vitamin D; therefore, findings were not available for this report.
- Because of the risk of toxicity, we do not recommend high-dose supplementation of vitamins A or D, particularly for women of childbearing potential *(146,149)*. The B vitamins (including folic acid) are relatively nontoxic *(53)* and, therefore, are low-risk supplements. Given the possibility that iron increases cancer risk *(45)*, we would advise against large doses of this mineral for the purposes of preventing cancer. No recommendations are possible regarding other minerals.

Americans need a strong message that there are many bioactive compounds in foods, especially in fruits and vegetables, which likely play an important role in the prevention of cancer and other diseases *(21,22)*. Dietary supplements cannot replace the benefits obtained from eating a diet high in fruit and vegetables, nor can they reverse the damage caused by a low-fiber, high-fat diet. Finally, health care providers should always ask patients about use of dietary supplements. As noted in this chapter, many Americans use multiple supplements on a regular basis. Clinicians must be aware of several issues including the potential for supplement–drug interactions, high monetary expenditures for supplements, and the tempting possibility for patients to replace (or substitute) important healthy behaviors, such as maintaining or losing weight, engaging in physical activity, eating a low-fat/high-fruit and -vegetable diet, and smoking cessation, with a dietary supplement pill. However, published evidence suggests that users of dietary supplements adhere to healthy lifestyle behaviors *(58)*. Research results that will be published within the next 10–15 yr will provide clinicians with information that may be useful in formulating public health recommendations about dietary supplements and cancer prevention.

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5 Soy Consumption and Cancer Prevention

A Critical Review

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KEY POINTS

- Soybeans contain several potential anticancer components.
- Epidemiological studies suggest that consumption of soy foods is associated with lower risk of breast and prostate cancer.
- The epidemiological evidence for the association of soy food consumption and reduced risk of other cancers is not consistent.
- Animal studies generally support the conclusion that some components in soy prevent the development and progression of breast, prostate, and colorectal cancers.
- In animal studies, it appears that soy may act synergistically with bioactive components from other foods (e.g., tea) to reduce breast and prostate cancer.
- There are several possible mechanisms by which soy bioactives may reduce cancer risk.

1. INTRODUCTION

Cancer chemoprevention refers to the reduction of cancer incidence by administration of agents or drugs that inhibit, reverse, or retard the cancer process. The role of soy-based foods or soy-based products as potential cancer chemopreventive agents has been an increasingly important subject of research. Since publication of the previous review on soy and cancer prevention in the second edition of *Preventive Nutrition*, more epidemiological investigations have been conducted. In addition, basic laboratory investigations using both in vitro and animal models have been conducted extensively to identify the bioactive components in soybeans, determine the efficacy of these components in prevention and/or treatment of cancer, and elucidate the underlying mechanisms of action by which these bioactive components exert their preventive activities. Although most laboratory studies have supported the role of soy products in prevention of certain forms of cancer, some studies suggest potential adverse effects of some soy components. A typical example is that animal studies suggest that soy isoflavone genistein stimulated the growth of estrogen-dependent MCF-7

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breast tumor in ovariectomized animals, which raises concerns that women with estrogen-dependent breast cancer (BRCA) or at high risk of developing BRCA should avoid consumption of soy-based products. These negative studies have particularly attracted the attention of the public and the scientific community. Many scientists and health professionals experience a dilemma in explaining the results and making any recommendations to the public and to their patients.

The objectives of this chapter are to update epidemiological and in vivo experimental studies published in the past several years regarding soy products and cancer prevention, to discuss possible in vivo mechanisms based on animal studies, and to draw some conclusions and recommendations based on the totality of the evidence. Special emphasis is placed on discussing, and hopefully clarifying, the role of soy products (especially genistein) in estrogen-dependent breast tumors.

2. THE ROLE OF SOY PRODUCTS IN CANCER PREVENTION

In the past several years, investigation of the role of soy products in prevention of BRCA, prostate cancer (CaP), and colorectal cancer (CRC) continues to be one of the priorities in cancer prevention research. In addition, data have emerged regarding soy products and prevention of other forms of cancer. In particular, more animal studies have been conducted to identify the bioactive ingredients in soy products to evaluate the in vivo efficacy. This section provides a concise but critical review of the scientific data published in the past several years.

2.1. Soy and Breast Cancer Prevention: Epidemiological Studies

The role of soy products and soy bioactive components in BRCA prevention has been studied extensively. The association between soy product consumption and reduced risk of BRCA originated from the perspective of international investigations. Risk of BRCA varies widely, with about a sixfold difference between the highest risk (typically seen in women from the United States and western Europe) and the lowest, usually found in women from China and Japan *(1)*. Asian women born in the United States or in Western nations have similar BRCA incidence rates as women who reside in those areas *(2)*. Japanese women who move to the United States tend to have higher incidence rates of BRCA than Japanese women who remain in Japan *(2)*. These data have led researchers to believe that environmental factors, such as diet and chemical exposures, play a large role in the development of BRCA.

This association has been further tested in epidemiological investigations and in vivo animal studies. Previous studies generally support the association between increased consumption of soy products and reduced risk of BRCA *(3)*. In the past 5–6 yr, data from both epidemiological investigations and experimental studies have continued to emerge. The epidemiological studies on soy products and BRCA that have taken place since 1997 are summarized in Table 1. Among recent descriptive epidemiological studies, intake of soy products was not significantly associated with the risk of invasive BRCA in California *(4)*, the risk of BRCA in women of Hiroshima and Nagasaki (two cities ruined by atomic bombs during the World War II in Japan) *(5)*, or the risk of BRCA mortality in an international ecological study *(6)*. One population-based study in Japan indicated that consumption of miso soup or soy isoflavones was significantly associated with reduced risk of BRCA *(7)*.

Table 1
Epidemiological Studies of Soy Consumption and BRCA Risk

		Result	
Study (Reference)	*Population*	*Significance*[a]	*Association*
Descriptive studies			
Horn-Ross et al., 2002 *(4)*	Cohort study, 111,526 subjects, 711 cases (invasive BRCA), California	None	Phytoestrogens and risk of invasive BRCA
Key et al., 1999 *(5)*	34,759 women in Hiroshima and Nagasaki, Japan, 427 cases	None	Tofu and BRCA risk
Nagata et al., 2000 *(6)*	International ecological study	None	Soy protein and the mortality of BRCA
Yamamoto et al., 2003 *(7)*	Population-based, 21,852 women in Japan, 179 cases	—	Miso soup and BRCA risk
		—	Isoflavones intake and BRCA risk
Case control studies			
Dai et al., 2001 *(16)*	Asian population, 1459 cases, 1556 age-matched controls	—	Soyfood and risk of ER^+/PR^+ BRCA
		None	Soyfood and BRCA risk in regular soy eaters
Dai et al., 2002 *(11)*	Chinese population in Shanghai, China, 250 cases, 250 controls	—	Urinary isoflavone levels and BRCA risk
Den Tonkelaar et al., 2001 *(8)*	Postmenopausal women in Netherlands, 88 cases, 268 controls	None	Urinary excretion of genistein and BRCA risk
Horn-Ross et al., 2001 *(9)*	Multiethnic population, 1326 cases, 1657 controls	None	Phytoestrogens and BRCA risk
		—	Soymilk and soy burgers and BRCA risk
Ingram et al., 1997 *(12)*	144 cases, 144 controls, Australia	—	Urinary equol levels and BRCA risk
		—	Urinary enterolactone and BRCA risk
Murkies et al., 2000 *(10)*	Postmenopausal women in Australia, 18 cases, 20 controls	—	Urinary daidzein and BRCA risk
		None	Urinary genistein and BRCA risk
Shu et al., 2001 *(13)*	Asian population, 1459 cases, 1556 age-matched controls	—	Tofu during adolescence and BRCA risk in both pre- and postmenopausal women
		—	Soy food (excluding tofu) during adolescence and BRCA risk in both pre- and postmenopausal women

(Continued)

Table 1 *(Continued)*

Study (Reference)	*Population*	*Result*	
		Significance[a]	*Association*
Wu et al., 2002 *(14)*	501 cases, 594 controls, Asian- American women in California	—	Tofu and BRCA risk
			Soy food during adult life and BRCA risk
		—	Soy food during both adolescence and adult and BRCA risk
Zheng et al., 1999 *(15)*	Chinese women in China, 60 cases, 60 controls	—	Urinary excretion of isoflavones and BRCA risk
Clinical intervention studies			
Maskarinec et al., 2003 *(17)*	Double-blinded, randomized, placebo- controlled, isoflavones and mammographic supplements (100 mg) and placebo	None	Isoflavones and mammographic density of breast

[a]—significant negative association, $p < 0.05$; None, no significant association.
BRCA; breast cancer.

Case–control studies demonstrated more consistent association between soy intake and BRCA risk. The studies were conducted in different regions and different populations around the world, and the soy consumption was assessed by dietary intake of soy-based food, soy isoflavones and/or isoflavone secretion. Soy consumption, measured as food intake or urinary excretion of soy isoflavones, was associated with either a nonsignificant effect *(8–10)* or significant reduction of risk of BRCA *(10–15)* and both estrogen receptor (ER)-positive and progesterone receptor-positive BRCA *(16)*. Consumption of some soy products such as tofu *(14)*, especially during adolescence *(13)*, or soymilk and soy burgers *(9)* was also associated with a significant reduction of BRCA risk. There has been no report regarding epidemiological studies of an adverse effect of soy consumption on BRCA risk.

Increased mammographic density has been suggested to be a predictor of BRCA risk. In a pilot intervention study, premenopausal women were randomized to receive either a daily 100-mg supplement of isoflavone or a placebo over 12 mo. Isoflavone supplementation did not significantly change either the size of the dense areas or the percent densities *(17)*. These results suggest that isoflavones do not exert an estrogenic effect similar to hormone replacement therapy on mammographic density.

2.2. Soy and Breast Cancer Prevention: Animal Studies

In addition to promising epidemiological studies, animal studies using different animal models have shown that supplementation of soy bioactive components have preventive activities in BRCA carcinogenesis, progression, and/or metastasis, as shown in Table 2. Chemical carcinogens such as 7,12-dimethylbenz[a]anthracene (DMBA), *N*-methyl-*N*-nitrosourea (MNU), *N*-nitroso-*N*-methylurea (NMU) or 2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridine (PhIP) were used to induce mammary carcinogenesis in animals. Tumor incidence, multiplicity, and/or latency were used as endpoint parameters. Although some studies did not show significant effects of soy components such as soy

Table 2
Animal studies of soy consumption and breast cancer (BRCA) prevention

Study (Reference)	*Animal models*	*Treatment groups*	*Results*	
			Significance	*Association*
Chemical-induced				
Appelt et al., 1999 *(18)*	SD rats, DMBA	SPI with three isoflavone levels	None	SPI/isoflavones and tumor incidence/ multiplicity
Badger et al., 2001 *(26)*	Female SD rats, DMBA	SPI (20%)	—	SPI and tumor incidence
Cohen et al., 2000 *(19)*	F344 rats, NMU	SPI (10,20%) Isoflavone-depleted SPI (10,20%)	None	Soy diets (protein or isoflavones) and tumor incidence, latency, multiplicity or volume
Constantinou et al., 2001 *(20)*	SD rats, DMBA	Genistein (0.02%), daidzein (0.02%), genistein and daidzein (0.01% each), SPIs (normal or depleted levels of isoflavones)	—	SPI and tumor multiplicity
			None	SPI and tumor latency or tumor incidence
			—	Daidzein and tumor multiplicity
			None	Isoflavones and tumor latency or incidence
Fritz et al., 1998 *(25)*	SD rats, DMBA	Genistein (0.0025%, 0.025%)	—	Genistein and tumor incidence
			None	Genistein and tumor latency
Gallo et al., 2001 *(21)*	SD rats, DMBA	Soy extract containing 12% isoflavones and 35% saponins (0.35,0.7%)	None	Soy and tumor incidence
			—	Soy (0.7%) and percent of poorly differentiated tumors
Gotoh et al., 1998 *(27)*	SD rats, NMU	Powdered soybean (2, 10%), Biochanin A (0.001,0.005%)	—	Soybean (10%) and tumor multiplicity
			—	Biochanin A and tumor multiplicity
			—	Biochanin A (0.005%) and tumor incidence
Hakkak et al., 2000 *(24)*	SD rats, DMBA	SPI (20%)	—	SPI and tumor incidence in F2 generation
			None	SPI and tumor multiplicity
Lamartiniere et al., 2002 *(22)*	SD rats, DMBA	Daidzein (0.025%)	None	Daidzein and tumor incidence

(Continued)

Table 2 *(Continued)*

Study (Reference)	*Animal models*	*Treatment groups*	*Results*	
			Significance	*Association*
Ohta et al., 2000 *(28)*	SD rats, PhIP	Fermented soymilk(10%), soy isoflavone mixture (0.02,0.04%) in a high- fat diet	—	Fermented soymilk and tumor multiplicity, volume and incidence
			—	Soy isoflavone mixture and tumor multiplicity
Zaizen et al., 2000 *(23)*	F344 rats, MNU	SPI (20%) Soy hypocotyls (1.5 and 5.0%)	+	Soy diets and tumor latency
			None	Soy diets and tumor incidence
Transgenic				
Jin et al., 2002 *(29)*	MMTV- neu/ErbB-2 transgenic mice	genistein(0.025%), daidzein (0.025%), Isoflavone mixture (equivalent to genistein at 0.025%)	+	Genistein, daidzein, or isoflavone mixture and tumor latency
Mizunuma et al., 2002 *(31)*	MMTV-germ-free (GF) mice their conventionalized (CV) mice	Biochanin A (0.02%), daidzein (0.02%).	—	Biochanin A and tumor incidence in CV mice
			+	Biochanin A and tumor incidence in GF mice
			None	Daidzein and tumor incidence in either mice
Yang et al., 2003 *(30)*	MMTV-neu/Her-2	Crude soy protein (277 μg daidzein/g diet and 214 μg genistein/g diet)	+	Soy and latency of mammary tumor development
Transplantable				
Allred et al., 2001 *(37)*	MCF-7, ovariectomized nude mice, estrogen-depleted	SPI with different levels	+	Genistein and MCF-7 tumor growth of genistein
Allred et al., 2001 *(36)*	MCF-7, ovariectomized nude mice, estrogen-depleted	Genistein (0.075%) and genistin (0.12%)	+	Genistein or genistin and MCF-7 tumor growth

Hsieh et al., 1998 *(34)*	MCF-7, ovariectomized nude mice, estrogen-depleted	Genistein (0.075%)	+	Genistein and MCF-7 tumor growth
Ju et al., 2001 *(35)*	MCF-7, ovariectomized nude mice, estrogen-depleted	Genistein (0.0125%-0.1%)	+	Genistein and MCF-7 tumor growth in a dose-dependent manner
Shao et al., 1998 *(39)*	MCF-7, estrogen-subcut-eneous maintained	Genistein	—	Genistein and MCF-7 tumor growth
Yan et al., 2002 *(33)*	BALB/c, 4526 murine mammary carcinoma cell line	SPI (10, 20%)	—	SPI (20%) and tumor volume and frequency of metabasis
Yuan et al., 2003 *(32)*	MDA-MB- 231, sc	Genistein combined polysaccharide (1%)	—	Soy product and tumor growth and final tumor weight
Zhou et al., 2004 *(38)*	MCF-7, orthotopic, estrogen-maintained	SPC(0.1,0.5%), Genistin (0.028,0.14%)	—	SPC or genistin and MCF-7 tumor growth

[a]—, significant negative association, $p < 0.05$; +, significant positive association, $p < 0.05$; None, no significant association.

SPI; soy protein isolate; DMBA; 7,12-dimethylbenz(a) anthracene; MNU; *N*-methyl-*N*-nitrosourea; NMU; *N*-nitroso-*N*-methylurea; PhlP; 2-amino-1-methyl-6-phenylimidazol [4,5-b] pyridine.

protein isolate (SPI) and soy isoflavones on tumor incidence *(18–23)*, multiplicity *(18,19,24)*, or latency *(19,20,25)*, other studies indicated that SPI and soy isoflavones significantly reduced tumor incidence *(24–28)*, multiplicity *(20,27,28)* or latency *(23)*. A soy extract with isoflavones and saponins also significantly reduced poorly differentiated mammary tumor induced by DMBA *(21)*.

Recently, a transgenic mouse model for BRCA, MMTV-neu/Her-2 transgenic model, was used to determine the effect of soy bioactive components on prevention of mammary carcinogenesis. Supplementation of soy isoflavones or isoflavone-containing crude soy protein significantly prolonged the latency period of mammary tumor development *(29,30)*. Biochanin A, an isoflavone that can be converted to genistein in vivo, also significantly inhibited tumor incidence in conventional mice *(31)*.

The transplantable tumor model is a widely used in vivo model to evaluate the efficacy of treatment and to study mechanisms of action. Yuan and coworkers *(32)* found that a genistein-containing soy polysaccharide significantly inhibited growth of estrogen-independent breast tumors. Yan and coworkers *(33)* found that SPI at 20% of the diet significantly inhibited the growth and metastasis of a murine mammary carcinoma.

2.3. Does Genistein Stimulate the Growth of Estrogen-Dependent Breast Tumors?

The controversy with soy and BRCA comes from the results derived from transplantable animal models using estrogen-dependent MCF-7 human BRCA cells in immune-deficient mice. Studies using an estrogen-depleted ovariectomized animal model showed that dietary genistein or genistein-containing soy protein stimulated the growth of estrogen-dependent MCF-7 tumors in vivo *(34–37)*. These results have been extensively publicized, and concerns have been raised regarding use of soy products by women with BRCA or women at high risk of developing BRCA.

We investigated the effects of genistin (the natural form of genistein present in soy) and the soy phytochemical concentrate (SPC; the soy phytochemical mixture present in soy) on the growth of MCF-7 tumors in estrogen-maintained intact animal models *(38)*. Both genistin and SPC inhibited the growth of MCF-7 tumors in a dose- and time-dependent manner *(38)*. In this study, we further demonstrated that the combination of SPC at a low dietary level with tea had a suggestive synergistic effect on inhibiting the growth of MCF-7 tumors, although SPC alone did not significantly inhibit tumor growth *(38)*. This study clearly indicates that one of the major reasons by which the Asian diet may have protective effects on BRCA may be by interactive actions of several types of foods or bioactive food ingredients. By using the similar estrogen-maintained intact animal model, Shao et al. *(39)* also demonstrated that genistein inhibited MCF-7 tumor growth in a dose-dependent manner.

Explanation and debate of these controversial results are focused on which animal models are more clinically relevant. Clearly, estrogen plays a critical role in the growth and development of estrogen-dependent BRCA. One of the mechanisms by which compounds such as soy isoflavones and surrogate ER modulators exert an estrogenic/anti-estrogenic effect is by competing with endogenous estrogens to bind ERs. Estrogens are present in certain levels in postmenopausal women, whose adipose tissue becomes their major source of endogenous estrogen *(40)*. Therefore, competitive binding by estrogens and genistein (or other phytoestrogens) to ERs is a common biological behavior in vivo. We propose that an animal model with maintained estrogen levels to support growth of

a MCF-7 tumor would have greater clinical relevance than one with depleted estrogen levels for evaluating the effect of genistein on the growth of estrogen-dependent MCF-7 breast tumors in vivo. Although estrogen levels in the blood of ovariectomized female mice are comparable to that in postmenopausal women, the growth of MCF-7 estrogen-dependent breast tumors in ovariectomized and intact animal models requires estrogen supplements *(41)*; removal of estrogen pellets stops tumor growth. Conversely, estrogen-dependent breast tumors develop and progress in postmenopausal women, indicating that estrogen levels in postmenopausal women are sufficient to support growth of estrogen-dependent breast tumors. In animals, growth of estrogen-dependent breast tumors requires higher circulating levels of estrogen than those required for growth of estrogen-dependent breast tumors in women. If this effect indicates that tumor-stimulating estrogen bioactivity in animals might be lower than that in humans, then clinically relevant animal models of estrogen-dependent BRCA should contain estrogen levels adequate to support tumor growth.

Similarly to the animal studies, we also propose that a cell culture system with a maintained estrogen level would be more relevant than one with a depleted estrogen level for evaluating the effects of estrogenic/anti-estrogenic compounds on estrogen-dependent BRCA cells. Indeed, although genistein showed estrogenic activity and stimulated proliferation of MCF-7 cells and enhanced expression of the estrogen-responsive *pS2* gene in an estrogen-depleted cell culture system, it inhibited estrogen-induced proliferation of MCF-7 cells *(38,42,43)* and *pS2* expression in an estrogen-maintained system *(42,43)*.

In summary, recent epidemiological studies generally have supported the association between increased consumption of soy products and a reduced risk of BRCA, and animal studies have suggested that some soy components prevent the development and progression of BRCA. However, some animal studies have suggested that the soy isoflavone genistein stimulated the growth of estrogen-dependent breast tumors, which raises the issue of whether women at high risk of developing BRCA and/or women with BRCA should avoid soy isoflavone supplementation. We have provided results, together with those of other groups, to demonstrate that for estrogen-dependent breast tumor, an estrogen-maintained animal model may be more clinically relevant than an estrogen-depleted animal model. Although the effect of genistein on estrogen-dependent breast tumors in women still needs to be investigated, our results suggest that genistein or soy phytochemicals inhibits the growth of estrogen-dependent breast tumors. Moreover, there is a lower level of concern regarding consumption of foods containing soy and increased risk of BRCA.

2.4. Soy and Prostate Cancer Prevention: Epidemiological Studies

In the past several years, additional epidemiological studies have been conducted to investigate the relationship between soy intake and CaP risk. The updated references are listed in Table 3. Among three descriptive studies, one cross-nation study showed that soy consumption was correlated with a significantly lower mortality rate of CaP *(44)*, and one study of Seventh-Day Adventist men in California showed that consumption of soy milk was associated with reduced risk of CaP *(45)*, whereas another ecological study in Japan did not show significant association between soy consumption and CaP risk *(6)*.

Recent case–control studies have provided supporting evidence on soy consumption and reduced risk of CaP. Two case–control studies in multiethnic groups in the United States and Canada *(46)* or in Canada alone *(47)* indicated that consumption of soy foods

Table 3
Epidemiological Studies of Soy Consumption and CAP Risk

Study (Reference)	*Population*	*Result*	
		Significance[a]	*Association*
Descriptive studies			
Hebert et al., 1998 *(44)*	A cross-national study	—	Soy products and CaP mortality
Jacobsen et al., 1998 *(45)*	Seven-Day Adventist men in California; 225 cases in 12,395 subjects	—	Soymilk and CaP risk
Nagata et al., 2000 *(6)*	Ecological study, Japan	None	Soy food and CaP risk
Case-control studies			
Akaza et al., 2002 *(49)*	Japanese population; 141 cases, 112 cancer-free controls	—	Daidzein metabolizer (equol producer) and CaP risk
		—	Daidzein metabolizer (equol producer) and poorly differentiated CaP
Kolonel et al., 2000 *(46)*	Multiethnic population in the United States and Canada; 1619 cases, 1618 controls	—	Soy food and CaP risk
Strom et al., 1999 *(48)*	83 Caucasian cases, 107 controls, United States	—	Daidzein intake and CaP risk
		None	Genistein intake and CaP risk
Villeneuve et al., 1998 *(47)*	Multiethnic population in Canada; 1623 cases, 1623 controls	—	Tofu and CaP risk

[a]—, significant negative association, $p < 0.05$; None, no significant association. CaP, Prostate cancer.

or tofu reduced the risk of CaP. Two other studies investigated the effect of soy isoflavones on CaP and found that intake of daidzein was associated with significant reduction of CaP risk *(48)* and that daidzein metabolizers (equol producers) significantly reduced the risk of CaP or poorly differentiated CaP *(49)*, although intake of genistein was not significantly associated with the risk of CaP *(48)*.

2.5. Soy and Prostate Cancer Prevention: Animal Studies

Animal studies using different types of animal models for CaP have been conducted in the past few years to identify the bioactive components in soybeans, evaluate the efficacy of the components, and elucidate the mechanisms of action (Table 4). In the DMBA-induced prostate carcinogenesis model, genistein and daidzein significantly inhibited multiplicity of the ventral prostate carcinomas *(50)*, and a soy isoflavone mixture significantly reduced tumor incidence *(51)*. In an MNU-induced prostate carcinogenesis model, SPI reduced the incidence of prostate tumors *(52)*, and genistein reduced the incidence of invasive prostate adenocarcinoma *(53)*.

Table 4
Animal Studies of Soy Consumption and CaP Prevention

Tumor types and study (Reference)	*Tumor and animal*	*Treatment groups*	*Results*	
			Significance	*Association*
Chemical-induced				
Kato et al., 2000 *(50)*	F344 rats, DMBA	Genistein (0.1%), daidzein (0.1%)	—	Genistein or daidzein and multiplicity of the ventral prostate carcinoma
Onozawa et al., 1999 *(51)*	F344 rats, DMBA	Soybean isoflavone mixture (0.01, 0.04%)	—	Soy isoflavones and tumor incidence
Pollard et al., 2000 *(52)*	Lobound-Wister rats, MNU	SPI (20%), soy meal (30%)	—	SPI and tumor incidence
			None	Soy meal and tumor incidence
Wang et al., 2002 *(53)*	Lobund-Wistar rats, MNU	Genistein (0.0025,0.025%)	—	Genistein (0.025%) and invasive adenocarcinoma
Transgenic/spontaneous				
Mentor-Marcel et al., 2001 *(53)*	TRAMP, transgenic	Genistein (100-500mg/kg diet) vs control	—	Genistein and development of poorly differentiated prostate tumor
Pollard et al, 2000 *(54)*	Lobound-Wister rats, spontaneous	SPI (20%), soy meal (30%)	—	SPI and tumor incidence
			None	Soy meal and tumor incidence
Pollard et al., 2001 *(55)*	Lobound-Wister rats, spontaneous	SPI (20%), soy meal (30%)	—	SPI and incidence of hormone-independent tumor
			None	Soymeal and tumor incidence
Transplantable				
Aronson et al., 1999 *(60)*	SCID mice, LNCaP, subcutaneous implantation	SPI plus isoflavone extract in high- and and low-fat diets	—	SPI plus isoflavone extract in low-fat diet and tumor growth
Bylund et al., 2000 *(59)*	BALB/c mice, LNCaP, s.c. implantation	SPI (22.5%) vs milk protein (28%)	—	SPI and tumor volume, tumor weight, and serum PSA

(Continued)

Table 4 *(Continued)*

Tumor types and study (Reference)	*Tumor and animal*	*Treatment groups*	*Results*	
			Significance	*Association*
Cohen et al., 2003 *(58)*	Copenhagen rats, AT-1 androgen independent cell, subcutaneous implantation	SPI (5, 10, 20%)	+	SPI (10, 20%) and tumor growth
Landstrom et al., 1998 *(57)*	Copenhagen-Fisher rats, Dunning R3327 rat prostate cancer cell, subcutanuous implantation	Defatted soy flour (33.3%) vs low-fat milk powder (28.1%)	—	Soy flour and timor incidence, tumor volume and tumor growth rates
Wan et al., 1999 *(61)*	Nude mice, LNCaP cells, orthotopic	BBI (1%), BBIC(1,2,3%)	—	BBI and tumor weight or tumor doubling time
			None	BBIC and tumor weight or tumor doubling time
Zhou et al., 1999 *(103)*	SCID mice, LNCaP, subcutaneous implantation	SPI (20%), soy phytochemical concentrate (SPC, 0.2%,1.0%), SPI plus SPC	—	SPI (1.0%) and tumor growth
			—	SPI plus SPC (0.2%) and tumor growth
			—	SPI plus SPC (1.0%) and tumor growth
Zhou et al., 2002 *(62)*	SCID mice, LNCaP, orthotopic implantation	Isoflavone- depleted SPI (20%), genistin (0.14%), SPC (0.5%)	—	Genistein and tumor growth
			—	SPC and tumor growth
			None	Isoflavone-depleted SPI and tumor growth
			—	SPC and metastasis to lymph nodes and lungs
Zhou et al., 2003 *(63)*	SCID mice, LNCaP, orthotopic implantation	SPC (0.5%)	—	SPC and tumorigenicity rate
			—	SPC and tumor growth

[a]—, significant negative association, $p < 0.05$; +, significant positive association, $p < 0.05$; None, no significant association.

BBl; Bowman-Birk inhibitor; BBlC; BBI concentrate; SPC; soy phytochemcial concentrate; SPI; soy protein isolate; DMBA; 7,12-dimethylbenz(a) anthracene; MNU; *N*-methyl-*N*-nitrosourea.

Lobound-Wister rats also develop prostate tumors spontaneously. This spontaneous tumor model has been used to determine the effect of soy isoflavones and soy proteins on prostate carcinogenesis and metastasis. SPI supplementation significantly reduced tumor incidence *(54)* and the incidence of hormone-independent tumors *(55)*. A soy meal did not have a tumor-inhibiting effect, suggesting that soy meal may contain other ingredients that may have adverse effects. The transgenic adenocarcinoma of the mouse prostate (TRAMP) model was also used, and one study showed that genistein significantly inhibited the development of poorly differentiated prostate adenocarcinoma in a dose-dependent manner *(56)*.

Transplantable prostate tumor models have been widely used to determine the effects of soy components on the growth and progression of prostate tumors. Defatted soy flours significantly reduced incidence and growth of Dunning R3327 prostate tumors in a rat model *(57)*. However, SPI stimulated growth of androgen-independent prostate tumors in rats *(58)*. Dietary SPI *(59)* or SPI with soy isoflavones in a low-fat diet *(60)* significantly inhibited the growth of androgen-sensitive human prostate LNCaP tumors. Bowman-Birk inhibitor (BBI), a soybean protease inhibitor, also significantly inhibited the growth of LNCaP tumor *(61)*.

We evaluated the effects of a series of soy components on the growth and metastasis of LNCaP tumors. By using a subcutaneous tumor model, we found that SPI (20%), SPC (1%), or SPI (20%) plus SPC (0.2% or 1%) significantly inhibited tumor growth. We further applied an orthotopic prostate tumor model to mimic interactions between cancer cells and stromal matrix in the prostate, and found that both genistin and SPC significantly inhibited the growth of orthotopic LNCaP tumor *(62,63),* and SPC significantly inhibited tumorigenicity rate *(63),* and metastasis to lymph nodes and lungs *(62)*, whereas the inhibitory effect of the isoflavone-depleted soy protein was not significant *(62)*. The combined effects between soy phytochemicals and tea also were evaluated. The combination of SPC and black tea synergistically inhibited prostate tumorigenicity, final tumor weight, and metastases to lymph nodes, and the combination of SPC and green tea synergistically inhibited final tumor weight and metastasis *(63).*Our studies indicate that soy isoflavones and soy phytochemicals are important soy bioactive components that can inhibit the growth of prostate tumors and that SPC might contain other components that have antimetastasis activity. Our studies also suggest that combinations of bioactive components from several food sources (such as tea) and soy may account for the cancer prevention activity of the Asian diet.

In summary, the mounting studies from both epidemiological and animal studies in the past several years further support the preventive role of soy products in CaP.

2.6. Soy and Colorectal Cancer Prevention: Epidemiological Studies

Some epidemiological studies have evaluated the association between soy food intake and the risk of CRC, and this topic has been reviewed *(64)*. In general, previous epidemiological studies did not provide sufficient evidence to support the protective role of soy food intake in CRC. Recent epidemiological studies are summarized in Table 5. In an ecological study, Nagata et al. *(6)* found a significant positive association between soy consumption and reduced CRC mortality in Japan. However, other case–control studies failed to demonstrate significant associations between soy-based foods or tofu intake and CRC risk *(65–67)*, except that soy-based food intake was associated with a reduced risk of CRC in women *(66)*. Overall, recent epidemiological studies do not suggest an association between soy-based food intake and reduced risk of CRC.

Table 5
Epidemiological Studies of Soy Consumption and CRC Risk

Study (Reference)	*Population*	*Result*	
		Significance[a]	*Association*
Descriptive studies			
Nagata et al., 2000 *(6)*	Ecological study, Japan	+	Soy foods and CRC mortality in both sexes
Case-control studies			
Seow et al., 2002 *(65)*	121 cases, 222 controls, Chinese population in Singapore	None	Soy intake and CRC risk
Le Marchand et al., 1997 *(66)*	Multiethnic population, United States; 1192 cases, 1192 controls	—	Soyfoods and CRC risk in women
		None	Soyfoods and CRC risk in men
Nishi et al., 1997 *(67)*	330 cases, 660 controls, Japanese	None	Tofu and CRC risk
Clinical intervention studies			
Bennink et al., 2001 *(68)*	"At risk" population, double-blind, SPI group (129 subjects), casein group (13 subjects)	—	SPI and labeling index
		—	SPI and proliferation zone

[a]—, significant negative association $p < 0.05$; +, significant positive association, $p < 0.05$; None, no significant association. CRC, colorectal cancer; SPI, soy protein isolate.

Despite nonsignificant associations between soy-based food intake and reduced CRC risk in epidemiological studies, a small clinical intervention study demonstrated that soy protein supplementation to a group of "at-risk" subjects significantly inhibited colon crypt cell proliferation, a suggested biomarker for colon cancer risk *(68)*.

2.7. Soy and Colorectal Cancer Prevention: Animal Studies

As shown in Table 6, chemical carcinogen-induced colon/rectal carcinogenesis models continue to be used as major in vivo systems to evaluate the effect of soy components on prevention of CRC. In the past several years, no animal study used a transplantable tumor model, and one study used a transgenic Apc (Min) mouse model. Sorensen and coworkers *(69)* compared the effect between a high-isoflavone SPI and a low-isoflavone SPI on colon carcinogenesis in Apc (Min) transgenic model and found that soy isoflavones did not significantly inhibit the incidence or multiplicity of tumor. However, it is unclear if soy protein had any protective effect in this model because a control group with another protein type was not included.

Azuma and coworkers conducted a series of animal studies using azoxymethane/deoxycholate-induced tumor models and found that a soybean high-molecular-weight fraction (HMF) isolated from the proteolytic digest of soy protein significantly inhibited tumor incidence *(70–72)* and especially tumor promotion by deoxycholate *(73)*. It was further shown that SPI significantly inhibited tumor incidence and the incidences of benign and invasive tumors *(74)*. Soya saponins *(75)*, full-fat soy flakes, defatted soy flour, or genistein *(76)* significantly inhibited multiplicity of aberrant crypt foci. However, Rao and coworkers

Table 6
Animal Studies of Soy Components and CRC Prevention

Tumor types and study (Reference)	*Tumor and animal*	*Treatment groups*	*Results*	
			Significance[a]	*Results*
Chemical-induced				
Azuma et al., 1999 *(73)*	F344 rats, Azoxymethane and deoxycholate	Soybean high- molecular-weight fraction (HMF) (26%)	—	Soybean HMF and colonic tumor promotion by deoxycholate
Azuma et al., 1999 *(70)*	F344 rats, Azoxymethane and deoxycholate	Soybean high- molecular-weight-fraction (HMF) (13.5%)	—	Soybean HMF and tumor incidence
Azuma et al., 2000 *(71)*	F344 rats, Azoxymethane and deoxycholate	Soybean HMF (13.5%) vs casein (10%)	—	Soybean HMF and tumor incidence
Azuma et al., 2000 *(72)*	F344 rats, Azoxymethane and deoxycholate	Soybean HMF (20.6%)	—	Soybean HMF and tumor incidence and development
Davies et al., 1999 *(124)*	F344 rats, Azoxymethane	High-isoflavone SPI vs low-isoflvone SPI	None	Isoflavones and tumor numbers
Gee et al., 2000 *(79)*	Wistar rats, 1,2-dimethyl-hydrazine (DMH)	Genistein (0.025%), SPI (containing 0.025% genistein)	+	Genistein or SPI before DMH and the incidence of aberrant crypt foci in distal colon
			None	Genistein or SPI after DMH and the incidence of aberrant crypt foci
Hakkak et al., 2001 *(74)*	SD rats, Azoxymethane	SPI (20%)	—	SPI and tumor incidence
			—	SPI and incidences of benign and invasive tumors
Kennedy et al., 2002 *(78)*	SD rats, DMH	BBIC(0.1,0.5%), BBI (0.01,0.1%), ABBIC (0.5%)	—	BBI or BBIC and tumor incidence
Koratkar et al., 1997 *(75)*	CF1 mice, Azoxymethane	Soybean saponins (3%)	—	Soya saponins and multiplicity of aberrant crypt foci

Continued

Table 6 *(Continued)*

Tumor types and study (Reference)	*Tumor and animal*	*Treatment groups*	*Results*	
			Significance[a]	*Results*
Rao et al., 1997 *(77)*	F344 rats, Azoxymethane	Genistein (0.025%)	None +	Genistein and tumor incidence Genistein and multiplicity of noninvasive and total adenocarcinoma
Thiagarajan et al., 1998 *(76)*	F344 rats, Azoxymethane	Soy protein concentrate (28.5%), Full-fat soy flakes (46%), Defatted soy flour (36%), genistein (0.015%) vs control	—	Full-fat soy flakes, defatted soy flour or genistein and number of colonic aberrant crypt foci
Transgenic/ spontaneous				
Sorensen et al., 1998 *(69)*	Apc(Min) mice	High-isoflavone SPI vs low-isoflavone SPI	None	Isoflavones and tumor incidence or multiplicity

[a]—, significant negative association $p < 0.05$; +, significant positive association, $p < 0.05$; None, no significant association.

BBI, BBIC ABBIC; autoclaved BBIC; SPI; soy protein isolate; DMBA; 7,12-dimethylbenz(a) anthracene; MNU; *N*-nitroso-*N*-methylurea; PhIP; 2-amino-1-methyl-6-phenylimidazol [4,5-b] pyridine.

(77) found that genistein did not affect tumor incidence but significantly stimulated multiplicity of noninvasive and total adenocarcinoma. By using another animal model of dimethylhydrazine (DMH)-induced aberrant crypt foci, Kennedy and coworkers found that soy protease BBI significantly inhibited tumor incidence *(78)*. However, Gee and coworkers *(79)* found that genistein or SPI treatment before, but not after, DMH induction significantly increased the incidence of aberrant crypt foci in the distal colon.

In summary, recent epidemiological investigations and in vivo animal studies, together with previous studies, provide suggestive but inconclusive evidence for a role of soy foods in prevention of CRC. More studies are needed to further determine the role of soy foods/soy bioactive components in prevention of CRC.

2.8. Soy and Prevention of Other Forms of Cancer: Epidemiological and Animal Studies

The association between consumption of soy foods and the risks of other forms of cancer has also been evaluated in several epidemiological studies in recent years. In addition, the efficacy of soy bioactive components was evaluated in several animal studies. Because of limited numbers of studies, this chapter reviews epidemiological and animal studies together. Epidemiological and animal studies are summarized in Tables 7 and 8, respectively.

Seven epidemiological studies investigated the association between soy food intake and the risk of stomach cancer, as shown in Table 7. In a prospective study, Nagata and coworkers *(80)* found that consumption of total soy products or fermented soy products was associated with a reduced risk of stomach cancer in Japanese men, and consumption of nonfermented soy products, but not total soy products, was associated with a reduced risk of stomach cancer in women. In another prospective study, consumption of tofu, but not soymilk, was associated with a reduced death rate of stomach cancer in Japan *(81)*. Two ecological studies showed a significant association between soy food consumption and a reduced stomach cancer risk or mortality *(6,82)*. In three case–control studies, one study showed a significant association between tofu intake and a reduced risk of postsurgery death from stomach cancer *(83)*; one study showed a significant association between intake of soy products and the reduced risk of stomach cancer in men *(84)*; but the other study did not show any significant association between soy and the risk of stomach cancer *(85)*.

Three case–control studies evaluated the association between soy food consumption and the risks of ovarian and endometrial cancer. Consumption of soy products or tofu was associated with a significant reduction of endometrial cancer risk *(86)*, and intake of soy isoflavones was associated with a significant reduction of endometrial cancer in postmenopausal women *(87)*. However, consumption of soy products was not significantly associated with the risk of ovarian cancer *(88)*.

Among five epidemiological studies of lung cancer, one ecological study *(6)* and one case–control study *(89)* failed to find significant associations between soy consumption and the risk of lung cancer. Consumption of soy foods was significantly associated with the risk of lung cancer in nonsmokers but was not significantly associated with a reduction of risk of lung adenocarcinoma in smokers *(90)*. Consumption of soybean curd significantly reduced the risk of lung adenocarcinoma, but not lung squamous and small cell carcinoma, in women *(91)*. Wakai et al. *(92)* found that soy food intake was associated with a significant reduction of lung cancer in Japanese men but not in women. They also

Table 7
Epidemiological Studies of Soy Consumption and Risks of Other Forms of Cancer

Study (Reference)	*Population*	*Result*	
		Significance[a]	*Association*
Stomach cancer			
Descriptive studies			
Nagata et al., 2002 *(80)*	Prospective study, 30,304 Japanese, 121 deaths	—	Soy products, fermented or nonfermented soy products and death rates from stomach cancer in men
		None	Soy products and death rate from stomach cancer in women
		—	Nonfermented soy products and stomach cancer death rates in women
Takezaki et al., 1999 *(82)*	Comparative ecological study, China 414 subjects from a high-risk area, 425 subjects from a low-risk area	—	Frequency of soy food consumption and gastric cancer risk in men and women
Nagata et al., 2000 *(6)*	Ecological study, Japanese, Japan	—	Soy foods and stomach cancer mortality
Ngoan et al., 2002 *(81)*	Prospective study, Japanese, Japan, 13250 subjects, 116 deaths	—	Tofu and death rate of stomach cancer
		None	Soymilk and death rate of stomach cancer
Case–control studies			
Huang et al., 2000 *(83)*	877 gastric cancer patients, Japanese, Japan	—	Tofu intake and risk of postsurgery death of stomach cancer
Ji et al., 1998 *(84)*	1124 cases, 1451 controls, Chinese, China	—	Soybean products and stomach cancer risk in men
		None	Soybean products and stomach cancer risk in women
Gao, et al., 1999 *(85)*	234 cases, 234 controls, Chinese, China	None	Soy product and stomach cancer risk
Ovarian and endometrial cancer			
Case–control studies			
Zhang et al., 2002 *(88)*	254 cases, 652 controls, China	None	Soybean products and ovarian cancer risk in women
Goodman et al., 1997 *(86)*	Multiethnic population in the United States, 332 cases, 511 controls	—	Tofu and endometrial cancer risk
		—	Tofu and other soy products and endometrial cancer risk

Horn-Ross et al., 2003 *(87)*	Non-Asian women in the United States 500 cases, 470 controls	—	Isoflavone intake and risk of endometrial cancer in postmenopausal women
	Lung cancer		
Descriptive studies			
Nagata et al., 2000 *(6)*	Ecological study	None	Soy consumption and lung cancer risk
Case-control studies			
Seow et al., 2002 *(90)*	Chinese women in Singapore, 303 cases, 765 controls	—	Soy foods and lung cancer risk and incidence of adenocarcinoma in nonsmokers
		None	Soy foods and lung cancer risk in smokers
Hu et al., 1997 *(89)*	Chinese population, China, 227 cases, 227 controls	None	Soy foods and lung cancer risk
Takezaki et al., 2001 *(91)*	Japanese, Japan, 1,045 cases, 4143 controls	—	Soybean curd and risk of lung adenocarcinoma in women
		None	Soybean curd and risk of lung squamous and small cell carcinomas in women
Wakai et al., 1999 *(92)*	Japanese, Japan, 333 cases, 666 controls	None	Tofu and risk of lung cancer or lung adenocarcinoma
		—	Tofu and risk of squamous cell carcinoma
		—	Soy foods and risk of lung cancer in men
		None	Soy foods and risk of lung cancer in women
	Thyroid Cancer		
Case–control studies			
Horn-Ross et al., 2002 *(93)*	Multiethnic population in the United States, 608 cases, 608 controls	—	Tofu and risk of thyroid cancer
		None	Other soy products and risk of thyroid cancer
Haselkorn et al., 2003 *(94)*	Asian population in the United States, 515 cases, 486 controls	—	Dietary isoflavone intake and risk of thyroid cancer
	Bladder cancer		
Descriptive studies			
Sun et al., 2002 *(96)*	Cohort study, 63,257 subjects, 62 cases, Singapore	+	Soy foods and risk of bladder cancer

Continued

Table 7 *(Continued)*

Study (Reference)	*Population*	*Result*	
		Significance[a]	*Association*
Case–control studies			
Lu et al., 1999 *(95)*	Chinese, Taiwan, 40 cases, 160 controls	None	Soy juice and bladder cancer risk
	Nasopharyngeal Cancer		
Case–control studies			
Ward et al., 2000 *(98)*	Chinese population in Taiwan, 375 cases 327 controls	None	Soy foods and risk of nasopharyngeal cancer
	Hepatic cancer		
Case–control studies			
Lei et al., 2000 *(99)*	Italian/Italy, 32 cases, 92 controls	—	Genistein intake and hepatic cancer
		None	Daidzein intake and hepatic cancer

[a]—, significant negative association, $p < 0.05$; +, significant positive association, $p < 0.05$; None, no significant association.

Table 8
Animal Studies of Soy Components and Prevention of Other Forms of Cancer

Tumor types and study (Reference)	*Animal model*	*Treatment groups*	*Results*	
			Significance[a]	*Association*
Transplantable				
Li et al. *(101)*, 1999	C57/BL mice, B16-BL6 murine melanoma cells intravenously	Genistein and daidzein	—	Genistein and daidzein and the number and size of lung metastases
Record et al. *(100)*, 1997	C57/BL mice, B16-BL6 cells, subcutaneously	Genistein(0.033%)	—	Genistein and tumor volume
Yan et al. *(102)*, 1997	C57/BL mice, B16-BL6 murine melanoma cells, intravenously	SPI (10, 15,20%) vs casein (20%)	—	SPI and the incidence and number of metastasis in lungs
Zhou et al. *(97)*, 1998	C57/BL6 mice, female, MB-49 murine bladder cancer cell line, subcutaneously	Genistein, SPC, SPI (20%)	—	Genistein, SPC, or SPI and bladder tumor volume

[a]—, significant negative association, $p < 0.05$.
SPC; soy phytochemical concentrate: SPI; soy protein isolate.

found that tofu intake was not significantly associated with the risk of lung cancer or lung adenocarcinoma but was associated with a significant reduction of squamous cell carcinoma *(92)*.

Two case–control studies examined the association between soy products and thyroid cancer. Tofu intake, but not other soy products, was associated with a significant reduction of thyroid cancer *(93)*, and intake of dietary isoflavones was associated with a significant reduction of the risk of thyroid cancer *(94)*.

One case–control study failed to find a significant association between soymilk intake and bladder cancer *(95)*. However, one cohort study found that soy food intake was associated with a significant increase of bladder cancer risk *(96)*. We studied the effect of soy components on bladder tumor growth in an animal model and found that genistein, dietary SPC, or dietary SPI all significantly inhibited the growth of bladder tumors in mice *(97)* (Table 8).

Two case–control studies evaluated the association between soy consumption and the risk of nasopharyngeal or hepatic cancer. Soy food intake was not associated with the risk of nasopharyngeal cancer *(98)*. Intake of genistein, but not daidzein, was associated with a significant reduction of hepatic cancer *(99)*.

Despite lack of epidemiological studies, three animal experiments studied the effects of soy components on melanoma (Table 8). Dietary genistein significantly inhibited the growth of the melanoma tumor *(100)*. Soy isoflavones or SPIs also significantly inhibited metastasis of melanoma tumor cells to lungs in the animal models *(101,102)*.

In summary, despite additional epidemiological and animal studies in recent years to determine the role of soy components in prevention of other forms of cancer, the data are neither consistent nor sufficient to suggest the role of soy components in prevention of these cancers. More epidemiological and animal studies are needed to determine the role of soy bioactive components in prevention of these forms of cancer.

2.9. Mechanisms of Action

Carcinogenesis involves a cascade of processes including initiation, promotion, progression, and metastasis. DNA damage caused by carcinogen exposure and reactive oxygen substances has been suggested to be one of the early steps in initiation of carcinogenesis, whereas deregulated cell proliferation, differentiation, and apoptosis and uncontrolled angiogenesis are major cellular modifiers involved in the promotion, progression, and metastasis stages of carcinogenesis. Both in vitro and in vivo animal models have been used to study the mechanisms by which soy bioactive components may have preventive activity on certain types of cancer. Among soy bioactive components, the soy isoflavone genistein has been studied extensively. A large body of data on mechanisms is derived from the in vitro studies, but only a few suggested mechanisms have been confirmed in the in vivo animal models. Although in vitro studies can provide important information on cellular and molecular mechanisms, there are substantial differences between in vitro and in vivo systems. Therefore, we review the mechanistic evidence that has been elucidated only in animal models. Because soy bioactive components may exert their preventive activities with different cancers via the similar mechanistic pathways, this review is made based on mechanistic pathways rather than cancer types.

Despite in vitro studies suggesting that the soy bioactive component genistein may have antioxidant activity and exerts its cancer prevention activities by modulating the initiation process, there is no in vivo evidence to support this suggestion. Animal studies

have focused mainly on the mechanisms by which soy bioactive components may prevent progression and metastasis processes of carcinogenesis. We focus on the cellular and molecular mechanisms by which soy bioactive components prevent progression and metastasis of carcinogenesis in vivo, mainly on cancer cell proliferation and apoptosis, and on tumor angiogenesis.

2.9.1. Modulation of Cancer Cell Apoptosis and Proliferation by Soy Bioactive Components

Apoptosis is an active process of programmed cell death designed to control cell populations in a variety of normal and neoplastic tissues. In cancer, it is regulated by numerous oncogenes, tumor suppressor genes, and growth factors. Some soy bioactive components such as genistein and SPI have been shown to induce apoptosis of cancer cells in vivo. SPI inhibited growth of prostate tumor and induced tumor cell apoptosis in vivo *(59)*. Antiproliferation activity of soy products has also been shown in a clinical intervention study. Bennink et al. *(68)* found that consumption of SPI significantly reduced the labeling index and proliferation zone of human colon mucosa.

We studied the mechanisms by which genistein, a soy phytochemical mixture, and soy protein inhibited the growth of bladder, breast, and prostate tumors in animal models. In the bladder cancer study *(97)*, we found that genistein, SPC, and SPI significantly inhibited tumor growth, associated in part with induction of apoptosis. All three components significantly inhibited tumor cell proliferation; SPC and SPI also significantly induced tumor cell apoptosis. In CaP studies, we found that soy protein or soy phytochemicals inhibited growth of LNCaP tumors associated with inhibition of cancer cell proliferation *(63,103)* and induction of cancer cell apoptosis in vivo *(62,103)*. Genistein also inhibited tumor growth and induced cancer cell apoptosis in vivo *(62)*. Genistein and SPC also prevented growth of estrogen-dependent breast tumors in animals, associated in part with inhibition of tumor cell proliferation *(63)*. Although SPC treatment alone did not significantly induce tumor cell apoptosis, its combination with tea resulted in a significant induction of tumor cell apoptosis *(63)*.

The molecular mechanisms by which soy protein and genistein induce tumor cell apoptosis and inhibit cell proliferation are still largely unknown. We determined p53 protein levels in prostate tumor samples and found that SPC treatment significantly increased p53 protein expression *(62)*. Because LNCaP cells express the wild-type p53, this result suggests that one of the molecular mechanisms by which soy phytochemicals inhibit prostate tumor growth is via induction of p53 expression and function. In vitro studies suggest that genistein may induce apoptosis and inhibit proliferation of cancer cells via modulation of expression of BCL-2, p21, and/or DNA topoisomerases, but they have not been verified in vivo *(104–109)*.

2.9.2. Modulation of Tumor Angiogenesis by Soy Bioactive Components

Angiogenesis, the generation of new blood vessels, is an essential step in tumor development, progression, and metastasis *(110)*. Angiogenesis is controlled by a series of angiogenesis and antiangiogenesis factors. Among these, vascular endothelial growth factor (VEGF)and basic fibroblast growth factor (bFGF) are believed to be important angiogenic factors.

The potential antiangiogenesis activities of soy bioactive components have been studied in our animal experiments. The prevention effects of genistein, SPC, and SPI on

growth of bladder tumors and prostate tumors were associated with significant inhibition of tumor microvessel density, a marker of angiogenesis in vivo *(62,63,97,103)*. Although genistein and SPC did not significantly reduce angiogenesis in MCF-7 tumors, the combination of SPC with tea significantly reduced angiogenesis *(38)*, which suggests that one possible mechanism by which soy and tea may synergistically suppress estrogen-dependent breast tumors may be via interactions that impede tumor angiogenesis. We also determined the molecular targets that may be responsible for anti-angiogenesis activity of soy components and found that the expression of bFGF, not VEGF, was slightly, but significantly, downregulated by soy protein and genistein in vivo *(62)*. Clearly, other angiogenic/anti-angiogenic factors are also responsible, and more mechanistic studies are needed to identify these target factors.

2.9.3. Soy Bioactive Components and Hormonal Modulation

Both BRCA and early stages of CaP are hormone-dependent. Clearly androgens and estrogens play important roles in the development and progression of these cancers. In the prostate, testosterone is rapidly and irreversibly converted to a more biologically active metabolite, dihydrotestosterone (DHT), by catalysis of 5α-reductase. Estrogen plays a key role in estrogen-dependent BRCA development and growth, exerting its effect on estrogen-dependent tumors by binding to ER-α and -β and inducing ER-dependent transcriptional expression of estrogen-responsive genes that promote cancer cell proliferation.

The role of soy bioactive components in estrogen metabolism has been a topic of active research, and several studies have been conducted to determine the effect of soy components on estrogens. Supplementation of soy products (soymilk, soy isoflavone mixture, or soy protein) either had no effect on serum estradiol levels in premenopausal *(111–115)* and postmenopausal women *(116,117)* or reduced serum estradiol levels in premenopausal *(118,119)* and postmenopausal women *(120)*. Soy products also resulted in a nonsignificant effect *(111–115,119)* or significant increase *(116,120,121)* of serum sex hormone-binding globulin levels. These results suggest that soy components may act by reducing estrogen bioactivity in human.

The data from animal studies on soy supplementation and estrogen metabolism are limited. Genistein reduced ER-α protein levels in MCF-7 cells in vitro *(122)*. We did not find significant modulation of either ER-α or -β in MCF-7 tumors by soy treatment *(38)*. However, the combination of SPC and green tea synergistically reduced ER-α protein levels in MCF-7 tumors *(38),* an outcome that suggests a possible mechanism for potent synergistic effects of the soy–green tea combination on estrogen-dependent breast tumors.

The effects of soy products on androgens in humans have not been adequately studied. Instead, animal studies have been conducted to determine the effects of soy components on androgen modulation. We found that dietary genistein significantly inhibited LNCaP prostate tumor growth and reduced serum levels of DHT and testosterone/DHT ratios *(62)*, suggesting that genistein may inhibit 5α-reductase in vivo. Soy phytochemicals did not significantly modulate serum levels of androgens *(62,63)*. However, the combination of SPC with green tea significantly reduced serum levels of total testosterone and DHT *(63),* suggesting that interactive modulation of androgen levels is one of the important mechanisms for the synergistic prevention of CaP progression by the soy–green tea combination. Studies by other groups indicate that dietary genistein downregulated androgen

receptor and ER-α and -β messenger RNA expression in the dorsolateral prostate in a dose-dependent manner *(123)*. However, soy products did not significantly modulate the expression of androgen receptor in LNCaP tumors in our study *(62)*.

3. CRITICAL ISSUES IN SOY AND CANCER PREVENTION RESEARCH

A growing body of evidence from both epidemiological and animal studies suggests that soybean may contain bioactive components that can prevent certain forms of cancer, such as BRCA, CaP, and possibly CRC. However, a large gap in data exists between animal and human studies that is a barrier to advancing the soy and cancer prevention hypothesis. Identification of the following critical issues will provide direction for future research in the area of soy and cancer prevention.

3.1. Identifying Bioactive Components in Soy

Soybeans contain a diverse array of components. Although some components are "bioactive" and the others are "inactive," it is possible that the presence of these active components may exert an additive or even synergistic action. It is also possible that some inactive components are required for the "active" components to be active. Process of soybeans results in concentration of certain types of bioactive components but may remove other bioactive and/or cofactors. Soy products used in clinical and animal studies contain a substantial variation in compositions, and the results of trials with different products are mixed. Well-characterized bioactive soy products need to be developed for clinical prevention study. Researchers should also clearly identify what they use in published studies. In addition, soy components may demonstrate different bioactivity for different forms of cancer or different subgroups of cancer. Therefore, different soy components may be more useful for prevention of specific types of cancer or phenotypes.

3.2. Application of Experimental Models for Evaluation

Successful identification of bioactive soy components requires application of appropriate testing systems, both in vitro and animal models. Although in vitro cell culture systems provide a fast and inexpensive screening system, it should be recognized that cell culture is an artificial system with different metabolic and biological processes from the in vivo system. Simple extrapolation of in vitro results to in vivo effects may be misleading.

Appropriate animal models are required to confirm any findings derived from the cell culture studies, identify other potential effective components, elucidate the in vivo mechanisms of action, and validate biomarkers that may be used as intermediate endpoints in future clinical trials. Bioactive components need to be validated and their toxicity and side effects determined in animal tumor models before any clinical investigations can be initiated. It is true that each animal model has its strengths and weaknesses. Therefore, it is important to use the most clinically relevant tumor models possible. For example, an orthotopic (intra-organ) tumor model may be more clinically relevant than a subcutaneous tumor model, because the orthotopic tumor model may mimic the tumor microenvironment and epithelial/stromal cell interactions in humans. In addition, the tumor behavior or biology may be a better parameter in judging the relevance of an animal model. For example, although the ovariectomized mouse has a similar level of blood estrogen as postmenopausal woman, estrogen-dependent MCF-7

breast tumors do not grow in the intact female mouse, suggesting that estrogen in the mouse may be less bioavailable to MCF-7 cells than that in patients with BRCA. Therefore, a clinically relevant animal model for estrogen-dependent human BRCA should provide a blood estrogen concentration at a level that can support tumor growth, as seen in patients with BRCA.

3.3. Effects of Interactions Between Soy Bioactive Components and Other Dietary Components on Cancer Prevention

The soy and cancer prevention hypothesis was originally derived from epidemiological observations that Asian populations have reduced risks of certain forms of cancer, and those migrating to the United States increase their risks of cancer development. In fact, several food items in the Asian diet have been suggested to be associated with reduced cancer risk, and soy consumption is one of these dietary factors. Therefore, it is possible that reduced cancer risk in the Asian population is the outcome of additive and/or synergistic effects among several dietary factors. Indeed, our animal studies indicated that soy phytochemicals and tea combination had a synergistic effect on preventing the growth of both androgen-sensitive LNCaP prostate tumors and estrogen-dependent MCF-7 tumors *(38,63)*. Unfortunately, few epidemiological investigations and animal studies have looked at the combined effects among dietary components on cancer risk. Because different bioactive components may target different sites in the process of carcinogenesis, combination of agents may lead to the development of a multiagent strategy to maximize the complementary effects of different agents. Future epidemiological investigations and animal studies to determine the combined effect of soy and other dietary components are needed.

3.4. Doses Needed for Laboratory and Clinical Studies

Another critical issue that needs to be resolved is the proper dose needed to display significant effects in clinical trials. Most animal studies found that higher doses of soy bioactive components than those are usually found in the Asian diet are required to achieve significant effects. Several reasons may explain this difference: (a) it takes longer for cancer to develop and progress in humans compared to animals, thus the higher doses may be needed to achieve the significant effect in animal studies; and (b) most animal studies only evaluate the effect of a single soy bioactive component, whereas humans consume other soy bioactive components and bioactive components from other foods. Therefore, it is not surprising that a higher dose of a single bioactive component may be required. The higher doses of a single soy component may be required in clinical intervention studies to achieve a significant effect.

3.5. Selection of Population for Intervention Trials and Intermediate Endpoints for Evaluation of Effectiveness

Although it would be ideal to select a normal population for intervention trials and use tumor incidence as the endpoint, such a study would be very costly and require a long period of intervention. Therefore, subjects that are usually at high risk of developing cancer are suitable candidates for intervention trials, and biomarkers that would reflect the cancer risk and respond to treatment should be used to evaluate the effectiveness of the intervention. However, caution is needed in selection of these subjects with different genetic backgrounds. Although they have similarly high risks of developing

cancer, the associated risk factors may be different. Soy bioactive components to be tested may target only one of these risk factors; therefore, their responses to the treatment may vary significantly.

Alternatively, cancer patients that have undergone treatments (chemotherapy, surgery, and/or radiation therapy) are another subgroup for secondary prevention trials to determine the effect of the intervention regimen on tumor recurrence and progression, and biomarkers that are related to tumor progression may be used as intermediate endpoints to evaluate the effectiveness of treatment. However, this type of intervention study may require even higher doses of bioactive components because of existence of the disease. The doses used in primary intervention trials to prevent the development of carcinogenesis may not be sufficient to achieve significant effects in secondary prevention trials to delay or reverse progression of tumorigenesis.

4. FUTURE RESEARCH

Identification of the critical issues in soy and cancer prevention allows us to propose several priorities in the future research.

4.1. Application of Animal Models to Identify the Effective Soy Bioactive Components

Despite limitations of each animal model, in vivo experiments using animal models that represent different types of cancer and different stages of carcinogenesis processes have contributed significantly to advances in cancer prevention research. Various types of animal models are needed to establish the safety and efficacy of soy bioactive components. Although pure soy compounds may have specific effects, a combination of several soy components may provide a more effective regimen for cancer prevention by targeting several pathways. More research emphasis should focus on identification of the soy component(s) for early prevention. Chemical carcinogen-induced carcinogenesis models are commonly used for this purpose. Several transgenic animal models, such as the MMTV/neu transgenic mouse model for mammary carcinogenesis *(29,30)* and the TRAMP model for prostate carcinogenesis *(56)*, have been used to evaluate the effects of soy components on prevention of these types of cancer.

4.2. Further Elucidation of Mechanisms and Identification and Verification of Biomarkers as Intermediate Endpoints

Despite progression in mechanistic research, the in vivo mechanisms by which soy bioactive components prevent carcinogenesis and tumorigenesis are largely unknown, and it is unclear what molecular events are responsible for inducing apoptosis and inhibiting proliferation and angiogenesis by soy bioactive components. Traditional methods of analysis of gene expression patterns have imposed a practical limit on the number of candidate genes. Highly parallel technologies exploiting sample hybridization to oligonucleotide or complementary DNA arrays permit the expression levels of tens of thousands of genes to be monitored simultaneously and rapidly. With the advent of DNA microarray technology, quantitative comparisons of thousands of genes can be made simultaneously. The application of this technology and/or other advanced technologies, such as proteomics, will greatly facilitate our mechanistic research and identification of biomarkers that could be used as intermediate endpoints.

4.3. Clinical Intervention Studies

With identification of effective bioactive components by animal studies, intervention studies are an appropriate consideration to assess the clinical effects of these components. The golden standard is still the randomized, double-blinded, and placebo-controlled intervention trial. Because of high cost and long duration of this type of trial, alternative trials with short and intermediate durations using smaller cohorts of a subgroup of patients with cancer may provide excellent and cost-effective approaches for efficacy testing. For example, patients with CaP prior to or following radical prostatectomy can be used to determine the efficacy of soy intervention prior to surgery. Of course, these trials focus on clinically significant cancer and only assess the effects of soy components on secondary prevention. However, these trials are essential in the development of effective cancer prevention strategy by using soy bioactive components.

5. CONCLUSIONS AND RECOMMENDATIONS

The epidemiological evidence to date supports the association between increased consumption of soy foods and reduced risks of certain forms of cancer, such as BRCA, CaP, and possibly CRC. Animal studies further indicate that soybeans contain several bioactive components that may provide the cancer prevention activity of soybeans. However, not all soy products are created equal. Different soy components may have different bioactivities, and processing procedures may substantially alter the composition of soy products. Much research is still required to determine whether a particular soy product or a soy extract is safe and effective.

Based on epidemiological observations, it is appropriate to recommend increased consumption of soy foods or whole soy-based products. However, it is not recommended to consume soy extracts or supplements without knowledge of their safety and effectiveness. In addition to increased consumption of soy foods, people should incorporate other healthy dietary habits and lifestyles, such as increased consumption of fruits and vegetables, reduced fat intake, increased tea drinking, and exercise. It is believed that supplementation of human diets with certain soybean products, together with a healthy dietary pattern and lifestyle, could markedly reduce the risk and the mortality rate of human cancers.

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6 Tomato, Lycopene, and Prostate Cancer

Jessica K. Campbell and John W. Erdman, Jr.

KEY POINTS

- Prostate cancer is the second leading cause of cancer death of men in the United States.
- Americans consume large quantities of tomatoes and tomato products, which contain various nutrients and phytochemicals, including lycopene.
- Epidemiological evidence strongly indicates a relationship between the increased consumption of tomatoes, tomato products, and lycopene and a reduced risk of prostate cancer.
- In vitro studies demonstrate that lycopene and other carotenoids decrease cancer cell proliferation in various cancer lines, including prostate cancer cells.
- In vivo animal studies indicate that consumption of a tomato powder diet delays chemically induced prostate cancer compared to the control diet.
- Lycopene is found in human tissues and serum and is the major carotenoid found in the human prostate.
- Plausible mechanisms of action for lycopene include acting as a singlet oxygen quencher, upregulating cellular communication, and modulation of xenobiotic metabolism.
- Because tomatoes contain a variety of phytochemicals in addition to lycopene, potential interactions and synergies may exist between these compounds to contribute to a reduction in prostate cancer.

1. INTRODUCTION

Prostate cancer (CaP) is the second leading cause of cancer death of men in the United States. In 2004, an estimated 230,000 new cases will be diagnosed and an estimated 29,900 deaths will occur in the United States *(1)*. Approaches for the prevention of CaP, such as nutrition and diet, are currently under intense investigation. Epidemiological evidence indicates that a diet rich in fruits and vegetables is associated with a lower risk of many forms of cancer *(2)*. Dietary components found in fruits and vegetables, such as fiber, vitamins, minerals, and phytochemicals, may act through specific or interactive mechanisms to provide cancer protection. The protective effects of tomatoes, tomato products, and the carotenoid lycopene are an ongoing focus of CaP research. This chapter initially reviews the composition of tomatoes and tomato products and then evaluates their potential relationship with a reduced risk of CaP. This chapter examines various in vitro and in vivo

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Table 1
Nutrient and Carotenoid Contents of Selected Tomato Products[a]

			Tomato product mg/100 g		
Nutrient	*Cooked tomatoes*	*Carotenoid*	*Canned tomatoes*	*Catsup*	*Sauce*
Folate (μg/100 g)	13	Phytoene	1.9	3.4	3.0
		Phytofluene	0.8	1.5	1.3
Potassium (mg/100 g)	279	ζ-Carotene	0.2	0.3	0.8
		Neurosporene	1.1	2.6	7.0
Vitamin A (IU/100 g)	743	Lycopene	9.3	17.2	18.0
		γ-Carotene	1.5	3.0	3.2
Vitamin C (mg/100 g)	23	β-Carotene	0.2	0.6	0.5

[a]Data from ref. *3*.

studies, as well as plausible mechanisms of action of lycopene on CaP. Finally, future research efforts in the area are suggested and patient recommendations are reviewed.

2. TOMATO CONSUMPTION AND COMPOSITION

Americans consume large quantities of tomatoes and tomato products; the consumption of these ranks second of all vegetables *(3)*. In 1995, a net total of 13.3 million tons of tomatoes were available for consumption, 85% of which were processed into products such as paste, puree, juice, and sauce and were either consumed or used as ingredients in other processed foods *(3)*. Because of this large production and consumption, tomatoes and tomato products serve as a ready and convenient way to provide necessary nutrients to humans.

Tomatoes contain various nutrients and phytochemicals. Tomatoes have varying concentrations of many essential vitamins and minerals, including folate, potassium, vitamin C, provitamin A carotenoids, and vitamin E *(3)*. The amounts of these nutrients along with the carotenoid contents in different tomato products are listed in Table 1. The major carotenoids include phytoene, phytofluene, ζ-carotene, neurosporene, lycopene, γ-carotene, and β-carotene. The red carotenoid, lycopene, has the highest carotenoid concentration in tomatoes and tomato products. Although other foods contain lycopene, such as watermelon and pink grapefruit, the concentrations of lycopene in these fruits are approximately one-half to one-fifth to that of canned tomatoes *(3)*. Therefore, tomatoes are the major source of lycopene in the American diet. Furthermore, tomatoes contain various flavonoids such as quercetin, kaempferol *(4)*, and naringenin *(5)*. Many of these phytochemicals and nutrients have antioxidant properties and, in combination with lycopene, may contribute to the health benefits of tomatoes and tomato products, as discussed later in the chapter.

3. BIOAVAILABILITY: FOOD PROCESSING AND THE PRESENCE OF LIPIDS

Lycopene is an open-chain hydrocarbon carotenoid containing 40 carbon atoms and 11 conjugated double bonds. It is found primarily (80–97%) in tomatoes and tomato

products as the all-*trans*-linear isomer *(6)*. Rotation around any of the conjugated double bonds allows formation of numerous possible *cis*-isomers of lycopene.

Lycopene intake is not always equivalent to the amount absorbed. Many factors, such as food processing, cooking, and the presence of dietary lipids, influence its availability for absorption. The release of lycopene from the fibrous food matrix is the first necessary step in its absorption. Cooking or food processing has been shown to increase the bioavailability of lycopene by aiding in its release from the food matrix into the lipid phase of the meal *(6)*. One study demonstrated that lycopene bioavailability from tomato paste was approximately three times greater than that of fresh tomatoes in humans, suggesting that processed tomato products may be more efficient sources of lycopene than raw tomatoes *(7)*. Similar to other carotenoids, lycopene is lipophilic and hydrophobic and, therefore, poorly absorbed without the presence of fat in the same meal. As demonstrated by Stahl and Sies *(8)*, tomato juice consumption, preheated in the presence of 1% corn oil, resulted in significantly increased total serum lycopene concentrations when compared to unheated tomato juice. Lycopene uptake by the enterocytes occurs through the formation of bile acid micelles, whose formation is stimulated by the presence of dietary fat. After being taken up by the enterocytes, lycopene is subsequently packaged with triglycerides and phospholipids into chylomicrons for transport into the mesenteric lymph and, ultimately, the portal blood *(9)*.

Although tomato products primarily contain the all-*trans* conformation of lycopene, numerous studies have shown that tissues and serum of humans and animals preferentially accumulate *cis*-isomers. Stahl and Sies *(8)* observed that serum from healthy human subjects contained 50% *cis*-isomers of lycopene after consumption of tomato juice that contained only 20% *cis*-isomers. In a more recent study, lycopene was found to exist in human serum and prostate in 18 different isomer forms; however, the sum of the *cis*-isomers accounted for the majority of the total lycopene *(10)*. In addition, our laboratory demonstrated the preferential absorption of *cis*-lycopene isomers using the ferret, an animal that absorbs carotenoids similarly to humans. In this study, ferrets were orally dosed with lycopene containing only 9% *cis*-isomers, yet mucosa, lymph, blood, and tissues all contained significantly more *cis*-lycopene isomers (approx 50%) than that of stomach or intestinal contents *(11)*. In vitro experiments suggest that *cis*-isomers are taken up more readily by lipid micelles and, therefore, more easily absorbed by enterocytes *(11)*.

4. EPIDEMIOLOGY

An abundance of epidemiological evidence suggests that a diet rich in fruits and vegetables is associated with a lower risk of several types of cancer. Earlier work by Block et al. *(2)* examined approx 200 epidemiological studies and found a positive relationship between higher intake of fruits and vegetables and reduced risks of some types of cancer, including cancer of the lung, colon, breast, cervix, esophagus, oral cavity, stomach, bladder, pancreas, and ovary. Consumption of fruits and/or vegetables provided statistically significant protection from various forms of cancer in 128 of 156 dietary studies. In fact, a twofold increased risk was observed at most cancer sites for those with a low intake of fruits and vegetables, after controlling for confounding factors *(2)*. However, in that review, the studies regarding CaP were inconclusive.

More recently, several epidemiological studies have specifically analyzed the relationship between consumption of tomatoes, tomato products, and lycopene and CaP risk (Table 2). Of particular interest is that of a large, comprehensive dietary prospective

Table 2
Summary of Selected Epidemiological Studies Evaluating the Role of Tomatoes, Lycopene, and Prostate Cancer Risk[a]

Publication (year)	*Study type*	*Subjects*	*Dietary variable*	*Risk*	*Statistical significance*
Giovannucci et al. (1995)	Cohort	47,894 Men 773 Cases	Dietary intake of tomato >10 servings/wk vs <1.5 servings/wk	RR = 0.65	Yes ($p = 0.01$)
			Raw tomato intake 2–4 servings/wk vs none	RR = 0.74	Yes ($p = 0.03$)
			Tomato sauce 2–4 servings/wk vs none	RR = 0.66	Yes ($p = 0.001$)
			Pizza 2–4 servings/wk vs none	RR = 0.85	Yes ($p = 0.05$)
Gann et al. (1999)	Nested case–control	14,916 Men 578 Cases 1294 Controls	Plasma lycopene quintiles >580.1 ng/mL vs <261.7 ng/mL	All cases OR = 0.75	No
				Aggressive cases OR = 0.56	Yes ($p = 0.05$)
Cohen et al. (2000)	Case–control	628 Cases 602 Controls	Cooked/raw tomato intake ≥3 servings/wk <1 servings/wk	Cooked tomato OR = 0.90	No
				Raw tomato OR = 1.22	No
Giovannucci et al. (2002)	Cohort	47,365 Men 2481 Cases	Lycopene intakes approx 18,780 µg/d vs approx 3415 µg/d	RR = 0.84	Yes ($p = 0.003$)
			Tomato sauce ≥2 servings/wk vs <1 serving/mo	All cases RR = 0.77	Yes ($p < 0.001$)
				Advanced cases RR = 0.65	Yes ($p = 0.02$)

[a]Data from ref. *16*. RR, relative risk; OR, odds ratio.

study by Giovannucci and colleagues *(12)*, which reported that high intake of lycopene was related to a statistically significant reduction of CaP risk by 21% when compared to the intake of carotenoids β-carotene, α-carotene, lutein, and β-cryptoxanthin. This study was composed of over 47,000 men from the Health Professionals Follow-up Study (HPFS) who were followed from 1986 to 1992 and periodically answered questionnaires concerning medical conditions, lifestyle, and diet. Participants answered a 131-item food-frequency questionnaire in 1986 and 1990. In this cohort, 773 cases of CaP were diagnosed by 1992. Statistical analysis found that of all fruits and vegetables included on the food-frequency questionnaire, raw tomatoes were significantly associated with a reduction of CaP risk (relative risk [RR] = 0.74; 95% confidence interval [CI], 0.58–0.93; *p*-trend = 0.03]. Furthermore, tomato products such as tomato sauce (RR = 0.66; 95% CI, 0.49–0.90; *p*-trend = 0.001) and pizza (RR = 0.85; 95% CI, 0.45–1.58; *p*-trend = 0.05) were also found to be statistically associated with reduced CaP risk when consumed two to four times a week compared to no consumption. When combining the tomato groups together, consumption of more than 10 servings of tomato products per week was related to a 35% reduction of CaP risk when compared to consumption of less than 1.5 servings per week (RR = 0.65; 95% CI, 0.44–0.95, *p*-trend = 0.01). Moreover, the protective effect from tomato products was greater for those with more aggressive or advanced stages of CaP (RR = 0.47; 95% CI, 0.22–1.00; *p*-trend = 0.03) *(12)*.

More recently, additional data from the HPFS was analyzed to verify if the association of frequent consumption of tomato products and/or lycopene with reduced risk of CaP would persist in this cohort. Of the 47,365 participants who completed dietary questionnaires in 1986, 1990, and 1994, 2481 men developed CaP by 1998 *(13)*. Data from 1986 through 1998 again showed that intake of lycopene was associated with reduced risk of CaP (RR for high vs low quintiles = 0.84; 95% CI, 0.73–0.96; *p*-trend = 0.003) *(13)*. Tomato sauce intake, when comparing two or more servings per week to less than one serving per week, was associated with a reduction in CaP risk (RR = 0.77; 95% CI, 0.66–0.90; *p*-trend < 0.001) and in extraprostatic cancers (RR = 0.65; 95% CI, 0.42–0.99; *p*-trend = 0.02). Additional data analyzed from this cohort of men confirmed the results found in the previous study: frequent consumption of tomato products is associated with a lower risk of CaP.

A different prospective study was designed to assess the relationship between plasma concentrations of several major antioxidants (including a variety of carotenoids and α- and γ-tocopherol) and CaP risk. This nested case–control study used plasma samples from men enrolled in the Physicians' Health Study, a randomized placebo-controlled trial of aspirin and β-carotene *(14)*. Subjects included 578 men who developed CaP and 1294 matched controls. Of the antioxidants measured, only plasma lycopene was significantly lower in cases of prostate cancer than in matched controls (*p*-trend = 0.04 for all cases) *(14)*. Furthermore, the odds ratios (ORs) for prostate cancers tended to decline with increasing plasma lycopene (fifth quintile OR = 0.75, 95% CI, 0.54–1.06; *p*-trend = 0.12), and a stronger inverse relationship for aggressive prostate cancer was seen (fifth quintile OR = 0.56, 95% CI, 0.34–0.91; *p*-trend = 0.05). Plasma lycopene was also strongly associated with lower risk of prostate cancer in the placebo group (fifth quintile OR = 0.40; *p*-trend = 0.006 for aggressive cancer) *(14)*. The results from this study coincide with those from the HPFS, which indicated that lycopene is positively associated with reduced risk of CaP and that increased intake of tomato products and other lycopene-containing foods may decrease CaP cases.

A recent case–control study examined the associations of fruit and vegetable intakes with CaP risk in more than 1200 men younger than age 65 yr from King County, Washington. Dietary categories included citrus fruits, cooked tomatoes, cruciferous vegetables, beans, and green leafy vegetables. Results demonstrated that men who ate at least 28 servings of vegetables per week had a 35% lower risk of CaP compared to men who ate less than 14 servings of vegetables per week (OR = 0.65; 95% CI, 0.45–0.94; *p*-trend = 0.01) *(15)*. No associations were found between fruit intake and CaP risk or for any specific category of fruits or vegetables, with the exception of crucifers. Interestingly, the consumption of three or more servings of cruciferous vegetables per week was associated with a 41% decreased risk of CaP compared to consumption of less than one serving per week *(15)*. No significant relationship was observed in this study regarding the consumption of cooked tomatoes. It should be noted that this study was composed of younger (<65 yr) patients with CaP , who may represent a unique subgroup with a specific genetic component leading to CaP. It was proposed that CaP occurrence at a younger age may characterize an accelerated cancer process as a result of genetic polymorphisms and may interact with dietary components differently than in the majority of patients with CaP (>65 yr) *(16)*.

Overall, epidemiological evidence—especially prospective trials—supports the hypothesis that tomatoes, tomato products, and lycopene may offer protection from CaP and validates the use of in vitro and in vivo studies to further investigate this association.

5. CELL CULTURE

In vitro studies have been used to analyze the relationship between tomatoes and lycopene and the reduction of CaP. The controlled environments for in vitro experiments allow for the investigation of the molecular and cellular effects of carotenoids on cancer cells in culture. Several carotenoids have been found to inhibit growth of a number of cancer cell lines, including prostate, melanoma, lung, mammary, colon, and leukemia *(17)*. Of the carotenoids, lycopene and β-carotene are the most commonly studied in cell culture models. Our laboratory reported that β-carotene at concentrations greater than 30 μmol/L significantly decreased growth of three different CaP cell lines, including PC-3, DU-145, and LNCaP cells *(18)*. In that work, ^{14}C-labeled retinol was detected in the CaP cells incubated with ^{14}C-labeled β-carotene, suggesting that the in vitro effects of β-carotene on CaP cells may partially result from the metabolic conversion of β-carotene (a provitamin A carotenoid) to retinol or other metabolites *(18)*. Pastori et al. *(19)* showed that lycopene at 1 μmol/L, when incubated with 50 μmol/L of α-tocopherol, inhibited CaP proliferation of PC-3 and DU-145 cells, although lycopene alone at concentrations of 5 μmol/L or less was not found to be a potent inhibitor. Furthermore, the effect of lycopene with α-tocopherol appeared synergistic, whereas similar results did not occur with treatments of lycopene with β-tocopherol or ascorbic acid *(19)*. Kotake-Nara et al. *(17)* recently evaluated 15 common food carotenoids regarding the growth of CaP cell lines PC-3, DU-145, and LNCaP. Their results demonstrated that lycopene, phytofluene, and ζ-carotene, all of which are present in tomatoes, significantly reduced cancer cell growth at concentrations of 20 μmol/L or less *(17)*. Although other carotenoids (including α-carotene, β-carotene, and lutein) decreased cancer cell viability, lycopene appeared to be the most potent acyclic carotenoid by significantly inhibiting CaP cell growth at as low as 5 μmol/L *(17)*. Studies by Levy et al. *(20)*

also demonstrated that lycopene was a more potent inhibitor of cell growth than α- and β-carotene in various human cancer cell lines including endometrial, mammary, and lung cancer cells. Proposed mechanisms for the inhibitory growth effects by carotenoids on cancer cells include cell-cycle arrest, enhancement of gap-junction communication, inhibition of malignant transformation, and the induction of apoptosis and cellular differentiation *(17)*. In addition, lycopene has been shown to inhibit insulin-like growth factor (IGF)-1 signaling in vitro *(20,21)*, which is relevant because high IGF-1 blood levels have been shown to be associated with a greater risk of CaP *(22)*.

6. ANIMAL STUDIES

Laboratory animal studies are also valuable in the elucidation of the mechanism by which lycopene or components of tomatoes may reduce CaP risk. Our laboratory has been very interested in the potential interrelationships of lycopene and androgen status *(23)*, because early CaP tumor growth is characterized by its androgen dependence *(24)*. This interrelationship was recently examined in our laboratory through the use of a rodent animal model *(23,25)*. In one study, male rats were fed one of four diets containing total lycopene concentrations of 0.00, 0.05, 0.50, or 5.0 g/kg. Androgen status of the rats was reduced in some groups through the use of surgical castration. As dietary lycopene increased (0.00–0.50 g/kg), serum and tissue lycopene concentrations increased significantly *(23)*. Furthermore, as dietary lycopene increased, the percentage of *cis*-lycopene in the liver increased. In addition, castrated rats accumulated twice as much liver lycopene and had a greater proportion of *cis*-lycopene isomers in the liver than the intact controls. Because androgens potentiate CaP, it is of relevance that androgen status is inversely associated with lycopene accumulation and apparently affects its metabolism *(23)*.

To further assess the role of androgen in altering hepatic lycopene accumulation, a second study was conducted in our laboratory using the same rodent model fed a diet containing 0.25 g/kg of lycopene *(25)*. In addition to the role of testosterone, the influence of 20% food restriction on lycopene metabolism was investigated. Results showed that despite a 13% lower dietary intake, castrated rats accumulated nearly twice as much hepatic lycopene as intact rats allowed to eat *ad libitum (25)*. Moreover, food restriction was found to significantly increase liver lycopene by 68%, despite a 20% decrease in lycopene intake *(25)*. The findings from this study imply that both excess calorie intake and higher testosterone levels may promote lycopene metabolism and degradation *(25)*. Additionally, our laboratory is interested in determining whether provision of lycopene and other tomato antioxidants will reduce the adverse effects of oxidative metabolism in the prostate.

A more recent study in our laboratory examined the question of whether a lycopene-containing diet (161 mg/kg) and/or a 10% freeze-dried whole tomato diet (providing 13 mg/kg of lycopene) could enhance the survival of rats with chemically induced CaP compared to a control diet *(26)*. In addition, the role of dietary restriction in cancer modulation was examined by subdividing each dietary group into either a 20% food restriction or *ad libitum* feeding 3 d postcarcinogenesis. The animal model employed was an *N*-nitroso-*N*-methylurea (NMU) androgen-induced rat model that causes tumor development in prostate lobes similarly to that of prostate tumor development in humans *(27)*. At age 6 wk, tumors were initiated by NMU and androgen, followed by promotion of tumor formation through testosterone implantation. Results demonstrated

that rats fed the tomato diet had a significantly lower risk of death from CaP compared to that of the controls, whereas the survival of the lycopene-fed group was comparable to that of the control group *(26)*. The proportion of deaths caused by CaP in the control, lycopene, and tomato powder groups were 80, 72, and 62%, respectively. In addition, 20% dietary restriction slightly reduced CaP mortality, and the proportion of rats that developed CaP in the control and restricted diets were 79 and 65%, respectively *(26)*. Overall, this study demonstrates that consumption of tomato powder can inhibit chemically induced prostate carcinogenesis and that components of tomato, in addition to lycopene, warrant investigation.

Another study examining dietary lycopene supplementation and cancer prevention involved lung cancer. In this study, lycopene supplementation was shown to be protective against smoke-induced lung carcinogenesis in ferrets *(28)*. As reviewed by Liu and coworkers, higher intake of lycopene is associated with a lower risk of lung cancer, and lung cancer risk is related to higher levels of IGF-1 and/or lower levels of IGF-binding protein 3 (IGF-BP3). Liu et al. examined the effects of lycopene supplementation on plasma IGF-1/IGF-BP3 levels, changes in lung histology, cell proliferation, and apoptosis in ferrets with or without cigarette smoke exposure for 9 wk. Two doses of lycopene were used in this study design, including a low dose that was equivalent to a human intake of 15 mg/d of lycopene and a high dose that was equivalent to a human intake of 60 mg/d of lycopene. Results showed that ferrets supplemented with lycopene and exposed to smoke had significantly lower IGF-1/IGF-BP3 ratios as well as higher plasma IGF-BP3 levels compared to those of the control group. Both lycopene doses inhibited smoke-induced squamous metaplasia and cell proliferation. In addition, both low- and high-dose lycopene supplementations prevented the downregulated apoptosis in the lungs that is normally induced by cigarette smoke. Lycopene concentrations increased in the plasma and lungs of ferrets treated without cigarette smoke in a dose-dependent manner, whereas lycopene concentrations were substantially lower in the plasma and lungs of ferrets receiving both lycopene supplementation and smoke exposure *(28)*. In addition, *cis*-lycopene isomers in the lungs increased with exposure to smoke *(28)*. Overall, the results of this study suggest that lycopene may protect against smoke-induced lung cancer through upregulation of IGF-BP3, promotion of apoptosis, and the inhibition of cell proliferation, which may help explain current epidemiological evidence correlating a reduction of lung cancer risk with the increased consumption of tomatoes, tomato products, and lycopene *(29)*.

7. HUMAN STUDIES

Presently, 25 carotenoids and 9 metabolites have been identified and characterized from human serum and breast milk *(30)*. Carotenoids and their metabolites have been found in several human organs such as the liver, lung, breast, cervix, prostate, colon, and skin in μg-ng/g of tissue *(30)*. It is of particular interest that lycopene is the major carotenoid found in the prostate *(10)*, but various other tomato carotenoids are also present, such as β-, γ-, and ζ-carotene, phytofluene, and phytoene, in ng/g concentrations *(30)*. In addition, 2,6-cyclolycopene-1,5-diols A and B, metabolites derived from lycopene, have been found in human serum and milk, liver, breast, colon, lung, skin, and prostate tissue, which may have specific biological importance *(30)*, as discussed in the next section.

If lycopene does alter prostate carcinogenesis, then it is necessary to determine if lycopene is present in the human prostate at biologically significant concentrations. In a study by Clinton et al. *(10)*, paired benign and malignant prostate tissues from 25 men (ages 53–74 yr) undergoing prostatectomy for localized CaP were analyzed for concentrations of lycopene, other carotenoids, and vitamin A. A wide variety of carotenoids were detected in human prostate tissue, but lycopene had the highest mean concentration (0.80 nmol/g +/– 0.08 nmol/g) compared to other carotenoids. Lycopene concentrations within the prostate ranged from 0 to 2.58 nmol/g, and the total carotenoid concentrations ranged from 0.75 to 5.0 nmol/g. In addition, analysis of geometric lycopene isomers in tomato products, serum, and prostate tissue was conducted through the use of high-performance liquid chromatography with a polymeric C30 reversed phase column. Results showed that all-*trans*-lycopene accounted for 79 to 91%, and *cis*-lycopene isomers accounted for 9 to 21% of total lycopene in tomato and tomato products *(10)*. Serum lycopene concentrations ranged from 0.60 to 1.9 nmol/mL, with 58 to 73% consisting of *cis*-lycopene isomers and only 27 to 47% consisting of all-*trans*-lycopene *(10)*. Interestingly, all-*trans*-lycopene accounted for only 12 to 21% of total lycopene, whereas *cis*-isomers accounted for the majority (79–88%) of total lycopene in benign and malignant prostate tissues. The *cis*-isomers were distributed among 12 to 14 peaks in human serum and 14 to 18 peaks in human prostate tissue *(10)*.

In a more recent study, van Breeman et al. *(31)* analyzed all-*trans*- and *cis*-lycopene isomers in human serum and prostate tissues after dietary supplementation with tomato products. In this study, 32 men with clinical stage CaP (T1 or T2) consumed a tomato sauce-based pasta dish daily that provided 30 mg/d of lycopene (83% of which was all-*trans*-lycopene) for 3 wk before prostectomy. Prostate tissue was removed from 11 individuals by needle biopsy before dietary intervention and by prostectomy after supplementation and was analyzed for total lycopene and lycopene isomers. Data from this study demonstrated that after dietary supplementation, total serum lycopene increased twofold, from 0.664 to 1.30 μ*M (31)*. Furthermore, total lycopene in the prostate tissue also increased threefold, from 0.196 to 0.582 ng/mg. These findings are interesting not only because dietary supplementation with tomato products produced an increase in prostate lycopene concentrations but because all-*trans*-lycopene accounted for only approx 12.4% of total lycopene in prostate tissue before dietary intervention with tomato sauce but increased to 22.7% after supplementation *(31)*. There was also a small, yet significant increase of 2.8% of all-*trans*-lycopene in serum after supplementation *(31)*. Data from this study further confirm that *cis*-lycopene isomers accumulate in the prostate; however, the relevance to carcinogenesis remains unknown.

8. PLAUSIBLE MECHANISMS

Several plausible mechanisms have been suggested for the protective health benefits of tomatoes and lycopene. As reviewed by Boileau et al. *(32)*, carotenoids present in foods have distinct antioxidant properties and can quench free radicals. The human body is subjected to a variety of reactive radical species, including hydrogen peroxide, superoxide anion, and singlet oxygen, which are made endogenously by the body through aerobic metabolism and by immune cells. These reactive species are capable of reacting with DNA, proteins, and lipids, and these reactions can cause degradation of these molecules *(32)*. The degradation of DNA, proteins, and lipids is potentially detrimental

to one's health and, over time, may lead to disease occurrence such as cancer and cardiovascular disease (CVD). Reactive oxygen and nitrogen species can also enter the body from exogenous sources such as dietary components and cigarette smoke, consequently producing harm. Carotenoids have been found to react with and inactivate these compounds and, therefore, protect cells from damage and potentially decreasing the risk of cancer and CVD *(32)*. Carotenoids are especially effective quenchers of singlet oxygen *(33)*. The quenching ability of carotenoids is related to the number of conjugated double bonds present within the carotenoid *(33)*. Compared to other common carotenoids, lycopene is the most efficient singlet oxygen quencher in vitro, with higher quenching rate constants than all other C40 carotenoids *(33)*. This increased reactivity of lycopene is believed to result from the presence of two additional conjugated double bonds in this carotenoid *(33)*.

Another way in which lycopene has been thought to affect carcinogenesis is through the modulation of gap junctions between cells. Nontransformed cells communicate with each other either through hormonal signaling or via gap-junctional communication. Communication between cells is crucial for cellular and tissue homeostasis, and modifications in this communication may allow uncontrolled cell growth and potential cancer development. Transmembrane proteins called connexins typically form gap junctions and allow formation of channels in between adjacent cellular membranes. Low-molecular-weight compounds are able to translocate between adjacent cells through these channels, thereby allowing for cellular communication and signaling *(32)*. Carotenoids, including lycopene, have been shown to upregulate gap junctions, specifically by increasing connexin expression and thus enhancing the opportunity for cellular communication *(34)*. Stahl et al. *(35)* recently tested lycopene and its potential oxidation product, acycloretinoic acid, to determine which was more effective regarding upregulation of gap junctions and stabilization of connexin messenger RNA in human fetal skin fibroblasts. Results demonstrated that both compounds increased gap-junction communication, but lycopene was more effective at a low, physiological dose (0.1 μ*M*) when compared to a higher dose (1.0 μ*M*) of acycloretinoic acid *(35)*. Because an increase of gap-junction communication is correlated with growth inhibition, it is hypothesized that this is one potential mechanism by which lycopene inhibits growth of precancerous cells. Further research is necessary to prove this hypothesis.

A third potential mechanism for lycopene is through the modulation of xenobiotic metabolism. Xenobiotic metabolism involves enzymes that serve to detoxify foreign compounds that enter the body. Phase I xenobiotic metabolizing enzymes serve primarily to add or uncover a specific functional group of the xenobiotic, thus potentially activating it. Often, phase I enzymes activate a procarcinogen into a carcinogen. Phase II enzymes further react with the xenobiotic to make them unreactive and more easily excretable into the urine. Moreover, phase I and II enzymes often are coordinated in that the product of one becomes the substrate of the other. Under ideal circumstances, efficient clearance of xenobiotics from the body would consist of increasing phase II enzyme activity while decreasing phase I activity.

Many dietary factors have been shown to alter the expression and/or the activity of these xenobiotic metabolizing enzymes. It is presently unclear whether carotenoids have a significant role in the modulation of xenobiotic metabolism (*see* review in ref. *32*). Recent work by Brienholt and coworkers *(36)* examined the dose–response of lycopene on specific xenobiotic metabolizing enzymes. Female rats were orally dosed with

lycopene, with doses ranging from 0.001 to 0.1g/kg of body weight per day for 2 wk. Results demonstrated that phase I enzymes, such as P450-dependent enzymes, were significantly induced in a dose-dependent manner at all doses of lycopene *(36)*. In addition, lycopene was also able to induce hepatic quinone reductase, a phase II enzyme, by approximately twofold at lycopene doses between 0.001 and 0.05g/kg of body weight *(36)*. Glutathione transferase activity, a second phase II enzyme, also was significantly induced at lycopene dosage of 0.1g/kg of body weight *(36)*. Overall, this study supports the finding that lycopene may be effective in the modulation of xenobiotic metabolism, therefore providing a possible mechanism of protection from cancer. However, more research is needed in this area.

9. CRITICAL ISSUES

9.1. Supplementation

In the United States, total carotenoid intake is approx 7 mg/d, and typical lycopene intake is about 2 mg/d *(37)*. Although phytochemicals, including carotenoids, have been shown to positively modulate markers for chronic diseases in various animal and human studies, a high intake of a specific dietary substance is not always advantageous. For instance, β-carotene supplementation at 20 mg/d *(38)* or 30 mg/d with 25,000 IU of vitamin A *(39)* both failed to demonstrate protective effects; in fact, this supplementation enhanced lung cancer incidence in smokers/asbestos workers in human intervention trials. This finding was demonstrated despite the epidemiological evidence suggesting that β-carotene intakes of 4 mg/d or greater are associated with a reduced risk of lung cancer *(40)*. The key difference was that the epidemiological evidence was based on intake of whole foods *(2)*, rather than highly bioavailable β-carotene supplements. For this reason, it is advised that instead of taking supplements, individuals should increase their fruit and vegetable intakes to raise the carotenoids ingested.

9.2. Other Dietary Components in Tomatoes

Results from epidemiological and animal studies suggest the importance of diets containing whole foods rather than just one dietary component (Table 2) *(16)*. As mentioned earlier, there are many nutrients and phytochemicals other than lycopene in tomatoes (Table 1). The presence of other tomato carotenoids, including phytoene, phytofluene, neurosporene, and γ-carotene, may also contribute to the reduced risk of CaP through similar mechanisms as lycopene, although little research has been done to examine their role. These carotenoids also have been found in human serum, milk, and various tissues, including the prostate *(30)*. In addition, polyphenols (such as quercetin, kaempferol, and naringenin) present in tomatoes exhibit anticancer properties, including antioxidant activities, antiestrogenic properties, and antiproliferative activites *(41)*. Preliminary in vitro studies in our laboratory demonstrated that quercetin, kaempferol, and naringenin, inhibit cancer cell proliferation in both LNCaP cells (human prostate carcinoma) and Hepa1c1c7 cells (mouse hepatic carcinoma) in a dose-dependent manner (10–50 μ*M*) *(42)*. It has also been suggested that a synergy may be present between protective compounds found within whole tomato foods. For example, data from our laboratory show that combination treatments (25–50 μ*M*) of quercetin, kaempferol, and naringenin produce additive and perhaps synergistic inhibition of growth in both LNCaP and Hepa1c1c7 cell lines *(42)*. As mentioned earlier, lycopene and α-tocopherol

appear to act synergistically in the inhibition of cancer cell growth in various human prostate cancer cell lines *(19)*. Future research is needed in both in vitro and in vivo models to examine the role of tomato phytochemicals, in addition to lycopene and the potential synergies between these compounds regarding the reduction of CaP.

10. RECOMMENDATIONS AND CONCLUSIONS

Recommendations from the American Cancer Society include the consumption of five servings of fruits and vegetables a day to lower the risk of various cancers *(1)*. By following this recommendation, a variety of chemopreventive phytochemicals and nutrients would be supplied through the consumption of whole food products, which cannot be duplicated by supplements. Furthermore, some dietary components found in whole foods may be beneficial when other compounds are ingested simultaneously. It is also reasonable to recommend five servings of tomato products per week to reduce the risk of prostate cancer, other carcinomas, and various chronic diseases *(16)*. Furthermore, the American Institute for Cancer Research advises a diet rich in fruits, vegetables, whole grains, and beans in combination with maintenance of a healthy weight and regular physical activity *(43)*. These recommendations will not only lower an individual's risk for cancer but also the risk of other chronic diseases such as CVD. Annual prostate-specific antigen blood tests and digital rectal examinations also are recommended for men age 50 yr or older or for those in a higher risk group who are age 45 yr or older *(43)*. Higher risk factors include: age greater than 50 yr, African-American descent, having a close relative with prostate cancer, a diet high in fat and low in fruits and vegetables, and obesity *(43)*.

Overall, epidemiological evidence suggests that a diet abundant in fruits and vegetables is associated with a lower risk of several types of cancer. Regarding CaP, epidemiological studies focusing on dietary assessment imply that the intake of tomatoes and tomato products seem to be associated with a reduced risk. Because lycopene is the most abundant carotenoid in tomatoes and several plausible mechanisms of anticancer action have been proposed, it is plausible that lycopene is one protective compound that contributes to a reduction in CaP. Tomatoes and tomato products contain a variety of other carotenoids and phytochemicals, but these compounds have received little research attention.

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III Cardiovascular Disease Prevention

7 Iron and Heart Disease

A Review of the Epidemiological Data

Christopher T. Sempos, Richard F. Gillum, and Anne C. Looker

KEY POINTS

- In 1981, Dr. Jerome Sullivan proposed the hypothesis that the risk of coronary heart disease (CHD) increases in a positive fashion as body iron stores increase. Nearly all of the research on this topic in humans has come through observational, epidemiological studies. Serum ferritin and other less precise measures of body iron stores have been used in those studies to test the hypothesis.
- Serum ferritin was not significantly related to risk of developing CHD in five of seven traditional cohort studies.
- Serum ferritin was not significantly related to having cardiovascular disease in 9 of 10 case–control and cross-sectional studies.
- Serum transferrin saturation was not significantly related to risk of developing CHD in six of seven cohort studies.
- In an experiment, 14 men who were heavy smokers donated 500 mL of blood three times over a 14-wk period and demonstrated reductions in serum ferritin and improvement in indices designed to measure low-density lipoprotein cholesterol oxidizability. The experiment has not been repeated.
- The presence of the Cys282Tyr mutation, which accounts for most of the cases of hemochromatosis, was found to be associated with CHD risk in three cohort studies but not in six case–control or cross-sectional studies—study designs that are considered to be scientifically weaker than the cohort study design.
- At present, the vast majority of the epidemiological data does not support the hypothesis that body iron stores are directly related to the risk of developing CHD.

1. INTRODUCTION

In 1981, Dr. Jerome Sullivan *(1)* proposed a new theory to explain the differences in coronary heart disease (CHD) incidence and mortality between men and women. He

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noticed that as men and women age, the gaps between them in both heart disease incidence and in body iron stores decrease *(2,3)*. Lower stores of iron levels in women are mostly caused by menstrual blood loss, and with menopause the differences in iron stores decrease. As a result, Sullivan theorized that body iron stores are directly or positively related to CHD risk (i.e., the higher one's body iron stores, the greater one's CHD risk). The hypothesis was largely ignored until the publication of results from Finland by Salonen et al. *(4)*, which showed that men with serum ferritin levels (a measure of body iron stores) at or above 200 µg/L had double the risk of having a heart attack. Since then, however, there has been intense interest in this topic. It is believed that iron might promote the atherosclerosis leading to CHD by catalyzing the oxidation of low-density lipoprotein (LDL) cholesterol *(5–10)*.

The purpose of this chapter is to examine and update the recent epidemiological data directly related to Sullivan's hypothesis *(11,12)*. Important related topics to CHD risk (e.g., the relationship of antioxidant nutrients, oral contraceptives, estrogen-replacement therapy, and menopause) and iron and myocardial reperfusion injury are not discussed.

2. SERUM MEASURES OF BODY IRON STORES

Serum ferritin is currently the best measure of body iron stores that is feasible to use in epidemiological studies *(13)*. It is a fairly sensitive indicator of changes in body iron stores in healthy individuals (e.g., those not suffering from an infection, inflammation, or cancer) as they moves through the stages of iron status from deficient to replete to iron overload. Less direct and sensitive measures of body iron stores are serum iron, total iron-binding capacity (TIBC), and transferrin saturation (TS), which is calculated as the ratio of serum iron to TIBC. TS is the best measure of circulating iron available to tissues and is considered to be a better measure of iron stores than serum iron or TIBC but is not as good as serum ferritin. Other common iron status measures are even less directly related to body iron stores (e.g., hemoglobin, hematocrit, and erythrocyte protoporphyrin). Hemoglobin and hematocrit are measures of the oxygen-carrying capacity and viscosity of blood.

As body iron stores increase, so do serum ferritin levels *(14,15)*. As a result, serum ferritin can be useful in detecting iron deficiency and overload. A serum ferritin level of less than 15 µg/L has been used as an indicator of iron deficiency in both men and women *(16)*. Separate upper limits have been suggested for adult men (400 µg/L), menstruating women (200 µg/L), and postmenopausal women (300 µg/L) *(16)*.

TS and serum iron levels also tend to increase as stores increase over the normal range, whereas TIBC levels tend to decrease as stores increase. The opposite trends occur as body iron stores decrease. TS is considered to be a good measure of body iron stores when they are at very high levels, as in homozygous hemochromatosis (TS >60%), or at depleted levels (TS <16%; i.e., iron deficiency). Within the normal range of TS (20–60%), it is not clear how well TS reflects body iron stores. Data from the third National Health and Nutrition Examination Survey (NHANES III; Table 1), conducted from 1988 through 1994, show that there does appear to be a positive correlation, albeit low, between the two measurements with the result that when levels of TS increase, levels of serum ferritin levels tend to rise as well, especially in women. The correlation between logarithmic-transformed serum ferritin and TS for men and women ages 45 to 74 yr was $r = 0.22$ overall, $r = 0.14$ for men, and $r = 0.25$ for women (Table 2).

Table 1
Geometric[a] Mean Serum Ferritin by Serum Transferrin Saturation Level[b]

	Men		*Women*	
Transferrin saturation (%)[c]	*n*	*Ferritin (μg/L)*	*n*	*Ferritin (μg/L)*
<16%	492	93	974	49
16–19%	567	119	789	66
20–29%	1513	145	1638	87
30–44%	1101	161	714	99
45–59%	183	186	104	117
60%	53	269	17	220
Total	3909	142	4236	75

[a]Within each transferrin saturation group, the serum ferritin data were transformed using natural logarithms. The antilog of the mean of the log-transformed distribution is the geometric mean value shown Table 1.

[b]US men and women ages 45 yr and older.

[c]Calculated as the ratio of serum iron (μmol/L) divided by total iron binding capacity (μmol/L).

(From Centers for Disease Control and Prevention/National Center for Health Statistics. Unpublished data from the third National Health and Nutrition Examination Survey, conductedin 1988–1994.)

Table 2
Mean Levels of Serum Transferrin Saturation[a] and Geometric[b] Mean Serum Ferritin by Age and Sex[c]

	Serum transferrin (%)		*Geometric serum ferritin (μg/L)*	
Age group	*Men (n = 7529)*	*Women (n = 8533)*	*Men (n = 7529)*	*Women (n = 8533)*
20+	29	24	135	49
20–29	31	25	110	33
30–39	30	24	147	37
40–49	28	23	146	38
50–59	28	23	148	73
69–69	28	24	146	95
70+	27	24	128	89

[a]Calculated as the ratio of serum iron (μmol/L) divided by total iron binding capacity (μmol/L).

[b]Within in each age and sex group, the serum ferritin data were transformed using natural logarithms. The antilog of the mean of the log-transformed distribution is the geometric mean value shown in Table 2.

[c]US men and women ages 20 yr and older.

(From Centers for Disease Control and Prevention/National Center for Health Statistics. Unpublished data from the third National Health and Nutrition Examination Survey, conducted in 1988–1994.)

Serum iron status measures also are affected by inflammation, cancer, and infection. Serum ferritin levels tend to increase in response to inflammation *(14–17)*, whereas TS, TIBC, and serum iron levels decrease *(18)*. For example, in response to a heart attack, ferritin levels are initially raised, whereas TS, TIBC, and serum iron levels decrease *(19,20)*. In the study by van der Schouw et al. *(21)*, serum ferritin levels returned to control levels 6 wk after the heart attack, whereas TS and serum iron levels continued to be depressed.

The NHANES III distributions of serum TS and ferritin by age and gender are shown in Table 2. Two recent papers discussed the important issues regarding monitoring iron status measures over time in the national surveys *(22)* and gave more details about iron status data from NHANES III *(23)*.

3. BODY IRON STORES AND RISK OF HEART DISEASE: THE EPIDEMIOLOGICAL DATA

3.1. Cohort Studies Based on Serum Ferritin

Cohort studies also are referred to as prospective, incidence, follow-up, and longitudinal studies. The question asked in cohort studies is: Do persons with the risk factor develop or die from the disease more frequently or sooner than those who do not have the risk factor? For example, are persons who have high serum ferritin levels more likely to develop CHD in the future than persons who do not? Because risk-factor exposure is measured prior to the onset of clinically apparent disease, cohort studies are considered to be the strongest observational study design in epidemiology *(24,25)*. Using various measures of body iron stores, a number of researchers have attempted to assess the validity of the hypothesis that CHD risk increases with body iron stores (Table 3).

As stated earlier, the theory was largely ignored until the publication of results from the Finnish Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) *(4)*. The study consisted of 1931 randomly selected men who were ages 42, 48, 54, or 60 yr and who were free of clinical symptoms of CHD at baseline. During an average of 3 yr of follow-up, 46 men had either a definite or possible heart attack as defined by electrocardiogram (ECG) or enzyme criteria. Five additional men who were admitted to the hospital for prolonged chest pain did not meet the criteria. The authors reported that the results were not substantively changed by including those men, and the published results were based on 51 heart attacks.

Salonen et al. *(4)* reported finding a statistically significant linear association between serum ferritin level and the risk of heart attack ($z = 2.64$; $p < 0.01$) after adjusting for possible confounding. Thus, as serum ferritin levels increased, so did the risk of heart attack. However, the more surprising finding was that men with a serum ferritin of 200 µg/L had a greater than twofold higher risk of heart attack compared to those with lower serum ferritin values. The difference was statistically significant (relative risk [RR] = 2.2; 95% confidence interval [CI] 1.2–4.0, $p < 0.01$). Again, the results were adjusted for possible confounding in a multivariate model. Additionally, they reported finding that compared to men with serum ferritin levels less than 200 µg/L, men with a ferritin of 200–399 µg/L had a nearly identical risk of heart attack as did men with ferritin levels of 400 µg/L *(4)*.

The study by Salonen et al. *(4)*, although based on a small number of heart attacks, was well-conducted. In a letter to the editor, they presented data indicating that the relationship was still significant after an average of 5 yr of follow-up and 83 heart attacks (RR = 2.0; 95% CI, 1.2–3.1; $p = 0.004$) *(26)*. However, they have been criticized for not adequately adjusting for inflammation *(27)* and because there was a negative correlation between age and serum ferritin *(28)*. Neither criticism appears to be entirely justified *(29,30)*. The authors reported that they found no correlation between serum ferritin and plasma fibrinogen (an acute phase protein) in the whole sample or with C-reactive protein in a subsample *(4,29,30)*. Moreover, the authors adjusted for blood leukocyte

Table 3
Serum Ferritin and Heart Disease: Cohort Studies

Study (reference)	*Sex*	*Sample size*	*Association*
Kuopio Ischemic Heart Disease Risk Factor Study (KIHD)[a]			
Salonen et al. *(4)*	M	1931	+
Salonen et al. *(26)*	M	1931	+
Tuomainen et al. *(31)*	M	197[b]	+
Magnusson et al. *(33)*	M	990	None
	W	1046	None
Stampfer et al. *(34)*	M	476[b]	None
Sempos et al. *(39)*	M/W	1376	None
Mänttäri et al. *(35)*	M	268[b]	None
Knuiman et al. *(40)*	M/W	1612	None
Frey and Krider *(36)*	M	298[c]	None
Kiechl et al. *(37)*	M/W	826	+
Aronow and Ahn *(38)*	M/W	577	None

[a]All three studies are from the same cohort: Kuopio Ischemic Heart Disease Risk Factor Study (KIHD). The difference between the two studies by Salonen et al. is that the first consisted of the results after 3-yr of follow-up and the second after 5-yr of follow-up of the same individuals. The third paper is a study on a subset of the cohort in the first two studies examining the transferrin/ferritin ratio and risk of heart attack.

[b]Nested case–control study (i.e., a case–control or retrospective study that is "nested" within a cohort or cohort study) *(32,42)*. Serum transferrin/ferritin ratio (Tuomainen et al. *[31]*), or ferritin (Stampfer et al. *[34]*, Mänttäri et al. *[35]*) were determined on frozen sera collected at the beginning of the study. Cases accrued during the follow-up period and controls were selected from the pool of individuals who were at risk of having CHD at the time a case was diagnosed. Because of the efficient sample design, only small sample sizes are required *(32)*.

count in their analyses *(4)*. More importantly, the association between serum ferritin and heart attack was not attenuated when the analysis was repeated after excluding heart attacks that occurred within the first 6 mo following blood collection. As stated earlier, serum ferritin levels increase after a heart attack but return to baseline levels within 6 mo after the heart attack *(21)*.

In a subsample of their cohort, the Salonen research group also reported that the ratio of transferrin to ferritin was positively related to CHD risk *(31,32)*. This is not surprising given the strong finding in the larger cohort. Moreover, because the three studies by their group were based on the same set of individuals from the KIHD cohort, we consider them as one study supporting the Sullivan hypothesis (Table 2).

The results from eight additional cohort studies *(33–40)* have been reported regarding the association between serum ferritin and CHD (Table 2). Only one study found an association between serum ferritin and CHD *(37)*. In that study, Kiechl et al. *(37)* reported that the 5-yr progression of carotid stenosis was significantly related to serum ferritin levels. The study consisted of 826 men and women ages 40 to 79 yr who were randomly selected from the population of Bruneck, Italy. Carotid atherosclerosis was assessed by repeated carotid ultrasound evaluation.

The authors further reported that changes in iron stores were associated with changes in the progression of carotid atherosclerosis in that decreases in stores were associated with decreased risk of progression, whereas increases in stores were associated with

increased risk. We have found no reports in which these interesting results have been replicated.

Of the studies that found no association between serum ferritin and CHD, five were cohort studies *(33–35,39,40)* in the usual sense, and two appear to be based on case-series *(36,38)*. The studies by Magnusson et al. *(33)*, Sempos et al. *(39)*, Stampfer et al. *(34)*, Mänttäri et al. *(35)*, and Knuiman et al. *(40)* all used slightly different procedures to analyze their results and address different aspects of the iron hypothesis.

In the study by Magnusson et al. *(33)*, 2036 men and women from Iceland, ages 25 to 74 yr, were followed for an average of 8.5 yr. During that time, 81 participants (63 men and 18 women) had a heart attack. In their multivariate models, Magnusson et al. included both serum ferritin (in normal units or logarithmic-transformed) and serum TIBC as *continuous* variables to test whether a statistically significant linear association existed between serum ferritin and risk of heart attack. Neither serum ferritin (RR = 0.999, 95% CI 0.997–1.001) or log ferritin (RR = 0.781, 95% CI 0.540–1.129) were significantly associated with risk of heart attack.

In contrast with the results of Salonen et al., Magnusson et al.'s results indicated that body iron stores as measured by serum ferritin were not associated with the risk of having a heart attack. The interesting finding was that TIBC was shown to have a significant negative association with risk of heart attack (RR = 0.95, 95% CI 0.92–0.98). That is, those with higher TIBC levels had a lower risk of heart attack. The authors' interpretation of the results for TIBC were very interesting. As we have stated, TIBC levels are inversely related to body iron stores, but compared to ferritin, it is a relatively weak index of stores. However, TIBC is also known to have antioxidant properties *(41)*. Given the recent results concerning the oxidative modification of LDL cholesterol and the reported protective effects of antioxidant nutrients, Magnusson et al. postulated that, "If iron increases the risk of coronary artery disease by oxidizing LDL, as has been postulated, a critical step in its pathogenic pathway would be the accumulation of free iron in the subendothelial space. The serum iron binding capacity might be a more reliable indicator of this accumulation of free iron in the vessel wall than the total iron stores (*33*, p. 107). Additionally, the authors felt that their results "support the concept that iron being an important transition metal might contribute to atherogenesis along with other classic risk factors, although arguing against the recent hypothesis that iron stores *per se* increase risk (*33*, p. 107)."

Sempos et al. *(39)* directly addressed the issue of a threshold at 200 µg/L. The data were based on the mortality follow-up of 1376 Caucasian men and women who participated in the second NHANES. The NHANES II was conducted from 1976 to 1980, with mortality follow-up through December 31, 1992. Serum ferritin levels were divided into four categories: (a) less than 50 µg/L; (b) 50–99 µg/L (reference level); (c) 100–199 µg/L; and (d) 200 µg/L. For Caucasian men and women, serum ferritin levels of 200 µg/L were not associated with increased risk of death from all cardiovascular diseases (CVDs) (men: RR = 0.7, 95% CI 0.3–1.6; women: RR = 2.0, 95% CI 0.6–7.3) or CHD (men: RR = 1.2, 95% CI 0.5–2.9; women: RR = 1.2, 95% CI 0.3–4.9) compared with the reference level. Furthermore, there was no association with heart attack death in Caucasian men (RR = 0.5, 95% CI 0.2–1.2); there were not a sufficient number of heart attack deaths to test the hypothesis in Caucasian women (n = 13).

The study by Stampfer et al. *(34)* also directly addressed the issue of a threshold at 200 µg/L. Using a nested case–control design *(32)*, 238 men participating in the US

Physicians Study had a heart attack during the period after the 1982 baseline. Stored serum for those men and for 238 controls matched for age and smoking status were analyzed for serum ferritin concentrations. After adjustment for other CHD risk factors, men with serum ferritin levels of 200 µg/L were not found to have a higher risk of heart attack (RR = 1.1, 95% CI 0.7–1.6).

Similar results were found in another nested case–control study by Mänttäri et al. *(35)*. The participants in this study were a subset of men from the Finnish Helsinki Heart Study—a randomized clinical trial of the lipid-lowering drug gemfibrozil. In that study, the authors examined whether the threshold for ferritin occurs at much lower levels than the 200 µg/L cutoff used by Salonen et al. *(4)*. Mänttäri et al. looked at the risk of developing CHD in two groups of men with serum ferritin levels of 43 to 84 µg/L or 85 µg/L compared with men with serum ferritin levels of 42 µg/L and found that risk was not different.

In a 17-yr follow-up of a cohort from Busselton, Western Australia, no association was found between serum ferritin and risk of CHD or stroke *(40)*. The cohort included 1612 men and women who participated in the 1981 Busselton Heath Survey. The association was assessed with ferritin divided up into gender-specific tertiles or as a continuous variable. The tertiles were: men—126 µg/L, greater than 126–233 µg/L, and greater than 233 µg/L; women—49 µg/L, greater than 49–122 µg/L, and greater than 122 µg/L. Therefore, the results for women do not directly address the 200 µg/L cutoff. The results for the association between serum ferritin as a continuous variable and CHD were: men—RR = 0.944, 95% CI 0.71–1.25; women—RR = 0.849, 95% CI 0.60–1.2.

In case-series studies, patients who receive a particular test or procedure are followed over time using cohort study methods to evaluate the association between the test result and an outcome. The information from such a study may be very useful in suggesting hypotheses or testing a current one. However, because the test or procedure was ordered for a particular medical reason that may be favorably or unfavorably related to the outcome of interest the results from those studies may be biased and thus cannot be considered as rigorous as the data from a standard cohort study *(38)*. The study by Frey and Krider *(36)* was based on a clinical case series of 298 men who had serum ferritin measurements taken at some point over a 10-yr period (mean: 5.2 yr) in a West Virginia medical practice. Over that follow-up period, 32 men had a heart attack. The authors reported finding no difference in mean serum ferritin levels between patients who did or did not have a heart attack. Nor was there any association between risk of heart attack and a serum ferritin level above 200 µg/L. Unfortunately, none of the results appeared to have been adjusted for age or for other CHD risk factors. Finally, in a small study (it is not clear if it is a defined cohort or a case series) of 577 men and women ages 62 yr and older, no association was found between serum ferritin and incidence of CHD based on 3 yr of follow-up *(38)*.

3.2. Cohort Studies Based on TS

The association between TS *(43–47)*, serum iron *(44,46,48,49)*, or TIBC *(33,44,46,47)* and CHD risk has been investigated in seven different cohorts (Table 4). Only the study by Morrison et al. *(48)* reported a significant positive association. In that study, 9920 men and women ages 35–79 yr were followed for approx 16 yr. During that time, 141 men and 83 women died of an acute myocardial infarction (MI) or heart attack. Persons with a serum iron level of 175 µg/L compared to those with a value of

Table 4
Serum TS and Heart Disease: Cohort Studies

Study (reference)	*Sex*	*Sample size*	*Association*
NHANES I Follow-up Study[a]			
Sempos et al. *(43)*	M	1345	None
	W	1750	None
Liao et al. *(44)*	M	1827	None
	W	2410	None
Baer et al. *(45)*	M	15,167	None
	W	31,765	None
Reunanen et al. *(46)*	M	6068	None
	W	6102	None
van Asperen et al. *(47)*	M	129	None
	W	131	None
Morrison et al. *(48)*	M	?	+
(serum iron)	W	?	+
Magnusson et al. *(33)*	M	990	–
(TIBC)	W	1046	None
Corti et al. *(49)*	M	1385	–
(serum iron)	W	2551	–

[a]The studies by Sempos et al. and Liao et al. are from the same cohort: The NHANES I Epidemiologic Follow-up Study. Because cases within the first 3 yr of follow-up were deleted in the study by Sempos et al., the sample size in their study is smaller than the sample size reported by Liao et al.

less than 120 μg/L were found to have a significantly higher risk of dying from an acute MI (men: RR = 2.18, 95% CI 1.01–4.74; women: RR = 5.53, 95% CI 1.69–18.12). However, there were only seven deaths among the men and three among the women for those with a serum iron in the highest category. Although the results are consistent with those from Finland, the small numbers of events in the highest category tend to make the results less certain. In contrast, Coti et al. *(49)* reported a significant inverse association between serum iron and CHD and cardiovascular death, that is, the higher a person's serum iron level, the lower there is his or her risk of death from CHD or CVD.

The studies mentioned earlier that found no association between iron status and CHD have been criticized for using TS as a measure of body iron stores *(26,50,51)* and for using the lowest levels of TS as the comparison group. However, while criticizing the heart disease studies that have not found an association, the proponents of the iron hypothesis have cited studies that demonstrated an association between TS and risk of cancer *(51,52)* or serum iron and risk of CHD *(52)* as evidence that high body iron stores generally increase the risk of both heart disease and cancer.

Clearly, if TS is an indicator of body iron stores in one setting, then it must also be an indicator in the other. Because persons with very low TS levels (<16%) as well as those with very high levels (>60%) are probably ill individuals, we agree that persons with the lowest TS levels may not be the most appropriate comparison group. To explore the effects of using the lowest TS levels as the comparison group, we reanalyzed the relationship between TS and risk of CHD *(11)* using methods previously described *(11,43)* and by dividing TS into six categories (<16, 16–19, 20–29, 30–44, 45–59, and 60%). For those new analyses, the TS category of 20–29% was used as the reference category. A

Table 5
Serum Ferritin and Heart Disease: Case–Control and Cross-Sectional Studies

Study (reference)	*Sex*	*Sample size*	*Association*
Case-control studies			
Duthie et al. *(55)*	M/W	225	None
Rengström et al. *(56)*	M	194	None
Moore et al. *(57)*	M/W	730	None
Van der Schouw et al. *(58)*	M/W	162	None
Eichner et al. *(59)*	M	457	None
	W	114	None
Endbergs et al. *(60)*	M	208	None
	W	67	None
Cross-sectional studies			
Aronow *(61)*	M	171	None
	W	406	None
Solymoss et al. *(62)*	M	225	None
	W	74	None
Rauramaa et al. *(63)*	M	206	None
Kiechl et al. *(64)*	M/W	431	+ (ages 40–59 yr)
		416	None (ages 60–79 yr)

TS of less than 16% was used to indicate iron deficiency, and a TS between 16 and 29% was at the low end of the normal range. The RRs of CHD for the TS less than 16, 16–19, 30–44, 45–59, and 60% categories were 1.09, 1.09, 1.01, 0.75, and 0.76, respectively, for men and 1.28, 1.18, 0.84, 0.97, and 0.86, respectively, for women.

None of the estimates were significantly different ($p > 0.05$) than the risk of CHD in the 20–29% TS category. Persons with TS levels between 16 and 19% appeared to be at equal or higher risk of CHD, whereas persons with TS levels between 30 and 60% appeared to be at a possibly lower risk of CHD than those with a TS level of 20–29%. As a result, changing the comparison group for TS does not appear to change the conclusion that those data do not support the hypothesis that body iron stores are positively related to CHD risk.

There have also been a few cohort studies that used TS to look at the association between iron status and risk of stroke and all causes mortality. A U-shaped association between TS and stroke was reported for Caucasian women *(53)* where those with a TS level less than 29% and those with a TS level of 44% had significantly higher risks of stroke compared to those with a TS level of 30–36%. However, no association was found for Caucasian men or African-Americans. Additionally, no association has been found between TS and all causes mortality *(11,49,54)*.

3.3. Case–Control or Cross-Sectional Studies

There has been a number of studies *(55–64)* that have used a case–control or cross-sectional study design (Table 5). All of the studies used serum ferritin as the measure of body iron stores.

Only one *(64)* of the studies reported a significant positive association between serum ferritin and CHD, and this association was found only in the youngest of two age groups examined (ages 40–59 yr).

Case–control and cross-sectional studies generally are not considered to be as rigorous as cohort studies regarding observational study design *(24,25,42)*. The problem with both types of studies is that both disease status (e.g., heart disease) and risk factor exposure (e.g., serum ferritin) are measured simultaneously, thus it is impossible to tell if risk factor exposure preceded the disease or was a consequence of it. For example, the disease itself may alter body iron stores or serum ferritin levels. Additionally, people who already know they are sick may change their behavior to alter their levels of body iron stores. As a result, with case–control and cross-sectional experimental designs, it is difficult to establish if the exposure led to the disease or vice versa.

Because serum ferritin levels rise in response to inflammation, infection, cancer, and heart attack, clinically apparent CHD should increase serum ferritin levels so that, more often than not, a false-positive association would be found between ferritin and CHD. The fact that only one of the studies in this category found no association is a somewhat stronger argument against the hypothesis. On the other hand, including persons with CHD in these studies who have changed their diets to lower their serum cholesterol levels would tend to produce false-negative results, because a reduction in the intake of meat and animal products may also reduce levels of body iron stores over time and, as a result, reduce serum ferritin levels. One way to evaluate this possibility is to assess the association between serum total and LDL cholesterol and CHD concurrently with the serum ferritin and CHD assessments. In six of the nine studies that found no association between serum ferritin and CHD *(55–54,60,62,63)* and in the one study that found a positive association *(64)*, LDL cholesterol was positively associated with CHD, suggesting that it is less likely that changes in behavior produced false-negative results.

3.4. Dietary Iron and CHD Risk

Salonen et al. *(4)* also reported that dietary iron intake was positively associated with the risk of a heart attack. Other researchers have not been able to corroborate this finding *(11,44,46,48,57,63,65–67)*, and to date, only one other study *(66)* has found a positive association between dietary iron intake and CHD risk.

Two studies reported an association between heme iron intake and risk of heart attack but not with total iron intake *(65,67)*, whereas a third study found no association *(46)*. In any event, it is not clear what a relationship between dietary heme iron and CHD would mean in the context of a discussion regarding the association between body iron stores and CHD risk. Dietary intake methods do not adequately capture long-term dietary patterns, nor do they reflect the influence of growth and development or the effect of menopausal status on body iron stores, all of which are important factors in determining body iron stores. Additionally, an association between dietary iron and CHD risk may be a marker for a high-fat, high-cholesterol diet or may be something totally unrelated to body iron stores. For example, in a study by Klipstein-Grobusch et al. *(67)*, heme iron intake was positively correlated with hypercholesterolemia, a clear marker for a diet high in saturated fatty acids and cholesterol, as well as with hypertension, current cigarette smoking, and diabetes.

To help reduce the potential for confounding factors, epidemiologists, such as the authors of the studies on heme iron intake and CHD risk, use multivariate models to simulate an experiment (*32*, pp. 271–281). By using multivariate models that adjust for possible confounding factors, it is possible to evaluate the association between the

exposure of interest and the outcome variable while holding all other variables in the model constant *(32)*. However, there are limits to modeling. The unfortunate fact is that confounding caused by correlation in intakes or metabolism cannot simply be reduced or eliminated by statistical modeling *(68–70)*.

3.5. Blood Donor Studies and Iron and Oxidized LDL Cholesterol

Blood donation has been hypothesized as a way to decrease body iron stores and reduce the risk of heart disease *(71,72)*. Clearly, frequent blood donation will reduce body iron stores and serum ferritin levels *(73)*. Although there has been no direct evidence that blood donation will lead to a reduction in CHD, two approaches have been used to address this issue.

An indirect way to test this hypothesis involved examining the risk of CHD in voluntary blood donors and nondonors in existing epidemiological studies. There have been three such studies *(74–77)* (the paper by Salonen et al. *(76)* is an expanded version of the earlier paper by Tuomainen et al. *(75)* and is not a separate study) as well as a study examining the effects of blood donation on cancer incidence *(78)*. The results were mixed. Meyers et al. *(74)* reported that blood donation was associated with a reduced risk of CHD in nonsmoking men but not in male smokers or in women, whereas Tuomainen et al. *(75,76)* reported a significant reduction in the risk of heart attack in the cohort of Finnish men from the KIHD Study, which originally reported an association between serum ferritin and risk of heart attack. Ascherio et al. *(77)* found no association between history of blood donation and incidence of heart attack or fatal CHD.

There are several concerns regarding these studies. The principal concern is that volunteers for blood donation generally are healthier than nondonors and that any association may be a result of some unmeasured selection bias *(79,80)*. The data from both studies clearly do indicate that the volunteers were healthier. For example, in contrast to the usual practice in cohort studies, Salonen et al. *(75,76)* did not eliminate persons with pre-existing clinical CHD at baseline from their analyses as they had done in their previous studies *(4)*. In their study, over one-fourth of the 2529 nondonors (26.3%) had pre-existing disease compared to 8.5% of the 153 voluntary donors. As a result, it is impossible to determine if voluntary blood donation was influenced by the presence of heart disease, and, as stated earlier, that bias cannot be reduced or eliminated by statistical analysis *(68–70)*.

The paper by Ascherio et al. *(77)* examined the issue of blood donation using 38,244 men from the Health Professionals Study who were free of CHD at baseline. During 4 yr of follow-up, there were 328 nonfatal heart attacks and 131 CHD-related deaths among the cohort. In the 1992 questionnaire, the participants were asked to report the number of times they had donated blood over the previous 30 yr. The categories for lifetime blood donation in the analyses were: 0, 1–4, 5–9, 10–19, 20–29, and 30 times. To validate the self-reported history of lifetime blood donation, the authors measured serum ferritin levels in stored blood samples that were collected in 1986 from a random sample of 123 men in the study. The authors found a negative association between the number of times blood was donated and the level of serum ferritin. That finding supports the general accuracy of the blood donation questionnaire.

However, no association was found with nonfatal heart attack or fatal CHD. In what might have been a more direct test of the hypothesis, Salonen et al. *(52)* used a Latin Squares design to determine the effects of donating 500 mL of blood three times over a

14-wk period on measures of non-HDL (very LDL plus LDL) cholesterol oxidizability in 14 men who were heavy smokers. The authors reported finding that serum ferritin levels were reduced by 44%, whereas the maximal oxidation velocity was decreased by 20% and the lag time to start oxidation was lengthened by 33%.

Although interesting, the results of Salonen et al., even if replicated, are uncertain *(81)*. Currently, the LDL oxidation theory is interesting and increasingly accepted *(9)*. However, a problem lies in the measurement of LDL oxidation *(5)*. LDL oxidizability usually is measured by using LDL and exposing it to oxidative stress. The question is whether such a marker of LDL oxidizability corresponds to the extent of oxidation in vivo and whether it predicts risk of CHD *(77)*. The results of the few epidemiological studies that have examined the association between LDL oxidizability and markers of atherosclerosis or CHD risk are mixed *(82–85)*. There are also mixed results concerning the association between autoantibodies against oxidized LDL and atherosclerosis *(86)*. Thus, the available markers for oxidized LDL cannot yet be regarded as valid predictors of the risk of coronary artery disease in humans *(82)*.

Putting aside the possible problems with the measurement of oxidized LDL, several observational studies have looked at the association between serum ferritin and LDL oxidizability *(85,87,88)*. No association was found in any of the studies. In fact, Craig et al. *(88)* reported that serum ferritin accounted for about 1.6% of the variability in measures of LDL oxidizability, whereas serum copper accounted for 21% of that variability. Serum copper also is considered as a possible catalyst for the oxidation of LDL *(12,89)*.

The results from two human feeding studies appear to be at variance with the hypothesis that there is a positive association between body iron stores and the susceptibility of LDL to oxidation *(90,91)*. In the first study, Derstine et al. *(90)* tested the oxidizability of the LDL cholesterol in the plasma samples of 77 men and women ages 20–65 yr who were participating in one of three feeding studies. No association was found between serum ferritin and the measures of LDL oxidizability. In the second study, 26 women, ages 19–47 yr, who had low hemoglobin and serum ferritin levels (i.e., low iron status) participated in a randomized double-blind two-period crossover study *(91)*. Participants were randomized to an iron supplement (50 mg of elemental iron per day) or placebo. They were then randomly assigned to an average American diet or a low-fat diet known as the Step 2 Diet *(92)*. The average American diet contained 36% of the energy intake (i.e., kcals) as fat, with 15% of the total kcals from saturated fatty acids. The Step 2 Diet contained 26% of energy from fat and 7% from saturated fatty acids. Both diets contained about 25 g of fiber per day. Each diet was consumed for 3 wk with a 2-wk washout period. Although iron supplementation resulted in an increase in serum ferritin levels ($p = 0.008$), there was no association between iron supplementation and LDL oxidative susceptibility as measured by the rate of oxidation or total dienes.

4. IRON OVERLOAD AND CHD RISK

Hemochromatosis is a genetic disorder that, in the homozygous state, leads to iron overload, serious illness, and early death—usually from liver cancer, other liver diseases, cardiomyopathy, or diabetes *(93)*. Persons with the heterozygous form of hemochromatosis are unlikely to develop iron overload. The concern is that although iron overload is uncommon, heterozygotes tend to have higher levels of body iron stores, as indicated

by higher serum ferritin and TS levels, than persons without the condition, and the heterozygous form of the disease is relatively common (a prevalence of 10–15%) *(94–96)*. Therefore, if the iron hypothesis is correct, then heterozygotes form a substantial pool of persons at increased risk of CHD.

The cysteine-to-tyrosine substitution on the hemochromatosis gene (*Cys282Tyr* mutation) is thought to account for most of the cases of hemochromatosis. There have been nine published studies examining the association between the presence of the *Cys282Tyr* mutation in an individual and the risk of heart disease *(97–105)*. Note that the studies on this topic have looked at the association between the presence or absence of a mutation and CHD risk and have not examinedwhether serum ferritin or some other marker of body iron stores was related to risk. Taking that into consideration, the three prospective cohort studies found an association between the presence or absence of a mutation and the risk of CHD, and the six case-control or cross-sectional studies did not.

Roest et al. *(98)* found that in Dutch women, heterozygotes for the *Cys282Tyr* mutation were 1.5 times more likely to die of a heart attack (95% confidence limit [CL] 0.9–2.5), 2.4 times more likely to die of a stroke (95% CL 1.3–3.5), and 1.6 times more likely to die of any CVD (95% CL 1.1–2.4). By the same token, Toumainen et al. *(99)* reported that Finnish male heterozygotes had a 2.3-fold higher risk of suffering a heart attack (95% CL 1.1–4.8). Although Rasmussen et al. *(100)* reported that men and women in the US ARIC study were found to have a 2.7-fold higher risk of incident CHD (95% CL 1.2–6.1), they also reported that the results were somewhat unstable because of a few subjects in some strata. As a result, they advised that their results should be interpreted cautiously).

All of the studies that reported finding no association between the presence of the *Cys282Tyr* mutation were either case–control or cross-sectional studies *(97,101–105)*.

Prospective or cohort studies are generally thought to be superior to those study designs because, as noted earlier, the exposure or risk factor precedes the event; however, for these studies, the limitation may not be as severe, because we are discussing the association between an inherited mutation and the risk of disease. However, cross-sectional and case–control studies are limited in that subjects must be in relatively early clinical stages of developing CHD or must have had nonfatal events to be included in those studies. The relationship of the mutation to fatal first events may be missed in designs that are not prospective in nature. Accordingly, the results from cross-sectional and case–control studies need to be evaluated cautiously.

The possible association between hemochromatosis and CHD is even further clouded by the fact that an elevated fasting level of serum TS is used as the presumptive diagnosis for the disease *(106)*. Therefore, it is unclear why serum TS hasn't been considered as a risk factor for CHD in observational studies more often. It may be that the persons with hemochromatosis metabolize iron somewhat differently from those who have higher TS levels but who do not possess the heterozygous hemochromatosis gene. If this is the case, it might explain why serum TS generally hasn't been considered as a predictor of CHD risk.

5. SUMMARY

In 1981, Dr. Jerome Sullivan *(1)* proposed that body iron stores are directly or positively related to CHD risk (i.e., the higher your body iron stores, the greater your CHD risk). The hypothesis was largely ignored until the publication of results from Finland

by Salonen et al. *(4)*, which showed a positive relationship between serum ferritin levels and risk of heart attack in men. Although a plausible hypothesis was proposed by Sullivan to define a role for iron in the development of CHD, possibly by catalyzing the free radical oxidation of LDL cholesterol, the vast majority of the epidemiological results published since the study by Salonen et al. *(4)* have failed to support the original hypothesis. Whether looking at the direct relationships between serum ferritin and CHD risk, serum ferritin and measures of atherosclerosis, serum TS and CHD risk, iron intake and CHD risk, serum ferritin and measures of LDL oxidizability, or iron overload and CHD risk, the results are the same: the data do not consistently support the hypothesis that body iron stores are a risk factor for CHD. Additionally, the effect of blood donation on serum levels of oxidized LDL remains an open issue, as does the issue of the relationship of heterozygous hemochromatosis to risk of CHD.

6. RECOMMENDATIONS

Sound clinical guidance and public health recommendations must be based on reasonably solid evidence that the issue being recommended is both safe and effective. Given the results to date concerning the iron hypothesis, there can be no doubt about the recommendations. Further research, including basic research and large-scale epidemiological studies, is needed to fully assess the association between iron status and the risk of CVD and other adverse outcomes. Presently, the currently available data do not support radical changes in dietary recommendations or screening to detect high levels of serum ferritin, nor do they support the need for large-scale randomized trials of dietary restriction or phlebotomy as a means of lowering iron stores *(107)*.

Finally, it must be remembered that there are currently a number of proven CHD risk factors, including high blood cholesterol, high blood pressure, cigarette smoking, obesity, diabetes, and lack of exercise, for which there are proven and effective guidelines and measures for decreasing CHD risk *(108–111)*. Until other measures are proven and established, it is our recommendation that the public focus on them rather than on unproven measures.

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8 Homocysteine, Folic Acid, and Cardiovascular Disease Risk

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KEY POINTS

- The balance of homocysteine and methionine in the body can be disturbed because of low folic acid, B_{12}, or B_6 intake or low activity of several enzymes under genetic control.
- In the United States, the plateau of maximal lowering of homocysteine that can be attained by folic acid fortification of enriched flour and grain products has not yet been achieved.
- Inferences from meta-analyses restricted to prospective observational studies consistently point to a small elevated risk of coronary heart disease (CHD) associated with higher levels of homocysteine.
- Similarly, an independent effect of homocysteine is found on the risk of cerbrovascular and peripheral vascular disease.
- Most evidence suggests an elevated risk of CHD in the relatively common homozygotes (approx 10%) for the methylene tetrahydrofolate reductase *C677T* polymorphism (i.e., those with two copies of the cytosine to thymine mutation at neucleotide 677), among those with borderline folate status.
- Trials for secondary prevention of vascular disease are expected to provide evidence concerning the preventive potential of lowering homocysteine.

1. INTRODUCTION

The interest in homocysteine as a potential modifiable risk factor for cardiovascular disease exploded in the late 1990s and the turn of the millennium and remains high. Homocysteine is an amino acid byproduct of methionine metabolism. Historically, the finding of thrombotic complications in the rare hereditary disease of homocystinuria led to the hypothesis that the carriers for this condition, who number about 1 in 200 of the general population and have a partial metabolic defect and slightly elevated homocysteine levels, might have an increased frequency of vascular disease. Therefore, many studies were performed to relate elevated homocysteine blood levels—genetic in origin or not—to vascular disease. The majority of these investigations showed an association of hyperhomocysteinemia with coronary artery disease (CAD), cerebrovascular disease, and peripheral vascular disease.

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This chapter reviews the metabolism of homocysteine, its regulation, and its measurement. We review the relationship between folate intake and homocysteine levels, both in the absence and presence of fortification of enriched flour and grain products with synthetic folic acid in the United States and Canada. A brief summary of studies is provided reporting the association between blood folate levels and homocysteine levels and of the additional roles of pyridoxine and cobalamin in reducing elevated homocysteine. A summary of formal meta-analyses and systematic information syntheses of the association between homocysteine and coronary heart disease (CHD) and other cardiovascular disease (CVD) follows. We then provide a brief summary of the clinical trials of folic acid and other B-vitamins as homocysteine—reducing agents in the secondary prevention of CVD. Finally, we summarize the current understanding of the role of homocysteine and folic acid on CVD risk and discuss the implications for public policy and further research.

2. HOMOCYSTEINE

2.1. Homocysteine Metabolism

When protein is digested, methionine, an essential amino acid, is released. Homocysteine, a sulfur-containing amino acid, is a product of methionine metabolism and is an essential intracellular metabolite. Under normal circumstances, methionine and homocysteine cycle metabolically, with a steady proportion of homocysteine being exported into the blood (Fig. 1).

Homocysteine can be metabolized via transsulfuration or remethylation *(1,2)*. Vitamin B_6 is important in homocysteine transsulfuration, whereas folate and vitamin B_{12} play principal roles in homocysteine remethylation. The condensation of homocysteine and serine to form cystathionine is catalyzed by cystathionine β-synthase, an enzyme widely distributed in mammalian tissues. This enzyme requires pyridoxal 5'-phosphate (the biologically active form of vitamin B_6) as a cofactor, and *S*-adenosylmethionine (SAM) is a positive allosteric effector in this reaction *(3)*. Remethylation of homocysteine to methionine is accomplished by two pathways in humans. One pathway is catalyzed by *N*-5-methyltetrahydrofolate:homocysteine methyltransferase (methionine synthase). This enzyme, widely distributed in animal tissues, requires 5-methyltetrahydrofolate (the main circulating form of folate in plasma) as the methyl donor and cobalamin (vitamin B_{12}) as a cofactor. Methionine synthase also requires SAM once every 1000 turns of the catalytic cycle *(4)*. Tetrahydrofolate produced during remethylation reacts with serine in a reaction catalyzed by B_6-dependent serine:glycine hydroxymethyltransferase to produce glycine and 5,10-methylenetetrahydrofolate. The enzyme methylenetetrahydrofolate reductase (which is riboflavin [B_2]-dependent) regenerates 5-methyltetrahydrofolate. SAM inhibits this reaction, acting as a negative allosteric effector *(3)*. In the second pathway, betaine acts as the methyl donor, and the active enzyme is betaine:homocysteine methyltransferase, also inhibited by SAM. This second pathway is confined to the liver; its quantitative significance in humans is not fully elucidated *(5)*. Thus, the amount of circulating homocysteine is tightly regulated by four B-vitamins: folate, B_{12}, B_6, and B_2.

Human cells and tissues have different potentials for metabolizing homocysteine. All cells have the potential for remethylation of homocysteine, but only a limited number of human tissues and organs have the transulfuration pathway available. Defects in either

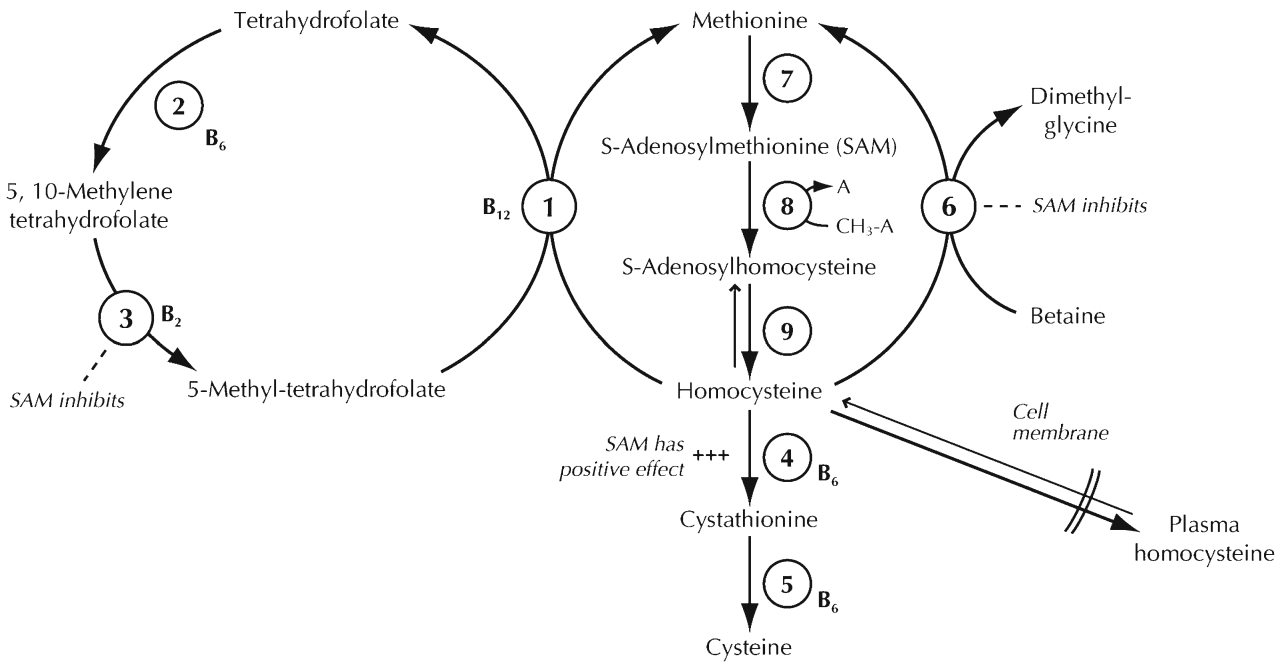

Fig. 1. Homocysteine metabolism in humans. Enzymes: 1. *N*-5-methyltetrahydrofolate: homocysteine methyltransferase (methionine synthase) [vitamin B_{12}], 2. Serine-glycine hydroxymethyltransferase [vitamin B_6]; 3. methylene-tetrahydrofolate reductase (MTHFR), [vitamin B_2]; 4. cystathionine β-synthase [vitamin B_6]; 5. γ cystathionase [vitamin B_6]; 6. Betaine: homocystenie methyltransferase; 7. Methionine adenosyltransferase; 8. Methyltransferases use SAM as substrate for methyl group transfers to acceptor (A) molecules; 9. *S*-Adenosylhomocysteine hydrolase.

pathway lead to intracellular accumulation of homocysteine, followed by an export to the circulation, thereby raising blood levels of homocysteine.

Homocysteine exists in plasma in several forms. In normal subjects, about 70 to 85% of the amino acid homocysteine is protein-bound via disulfide linkages, primarily to albumin *(6,7)*. The remaining homocysteine in plasma rapidly oxidizes to the disulfides homocystine (two homocysteine molecules linked together) and cysteine-homocysteine. The amount of total plasma/serum homocysteine is the sum of these three components and is referred to as homocysteine or total homocysteine (tHcy) *(8,9)*.

Even when the various methods for measuring tHcy correlate well (r = 0.95 and r = 0.98) with each other *(10,11)*, values for tHcy may vary somewhat depending on the laboratory and collection methods. Some variable changes in tHcy have been observed postprandially *(8)*; therefore, it is recommended to obtain fasting samples when possible. Fasting levels between 5 and 15 μmol/L are considered normal. Elevated levels of tHcy are referred to as moderate, intermediate, and severe hyperhomocysteinemia for concentrations between 16 and 30, between 31 and 100, and greater than 100 μmol/L, respectively *(7)*.

Shimakawa et al. *(12)* reported that among men and women in the Atherosclerosis Risk in Communities Study, methionine and protein intake did not show any significant association with plasma tHcy. However, a single dose of methionine administered as 100 mg/kg of body weight (i.e., about four times the dietary intake of a normal Western diet) *(13,14)* has been used as a diagnostic test to detect disordered homocysteine metabolism. Fasting and postmethionine load (PML) levels of tHcy have been found to be highly interrelated *(14,15)*. Elevated levels of fasting tHcy are believed to reflect folate- and cobalamin-dependent remethylation, whereas elevated PML is dependent on pyridoxal 5'-phosphate-dependent transsulfuration *(16)*.

2.2. Abnormalities of Homocysteine Regulation: Genetic Effects

The balance of methionine and homocysteine in the body can be disturbed because of inadequate intake of folic acid, B_{12}, or B_6 or because of genetic defects leading to low or absent enzyme activity rates in the relevant metabolic pathways. The finding that there is a strong genetic influence on homocysteine levels is supported by twin studies *(17,18)*, whereas familial studies also point to a role of shared environment *(19,20)*. An interruption of the transsulfuration or remethylation pathways of homocysteine (Fig. 1) produces hyperhomocysteinemia. Severe hyperhomocysteinemia (>100 μmol/L) is most often the result of homozygous cystathione β-synthase deficiency and leads to a condition called homocystinuria, which is characterized by an overaccumulation of tHcy, as evidenced by urinary excretion of homocysteine. The frequency of this genetic disorder in the US population has been estimated as 1 in 291,000 *(2)*, with 0.5% of the population being carriers or heterozygotes for cystathione β-synthase deficiency. Several other autosomal recessive inborn errors of metabolism can cause homocystinuria. These are also very rare and include five different vitamin B_{12} metabolic defects (CbC, D, E, F, and G) *(21)*. A homozygous deficiency of the enzyme, methylenetetrahydrofolate reductase (MTHFR), presents with neurological symptoms but is extremely rare and occurs in the general population at a rate of about one-tenth that of cystathione β-synthase deficiency *(22)*.

In 1988, Kang et al. *(23)* described a less severe defect of the MTHFR enzyme. This thermolabile variant of the MTHFR enzyme was associated with raised levels of tHcy.

This form occurs in neurologically normal subjects and is inherited as an autosomal recessive trait at an allele frequency of 5%, implying that 35% of the general European population (including people of European descent in the United States) are heterozygous for this trait. The elevated levels of tHcy seen in individuals with MTHFR enzyme variant in the homozygous state have been found to be correctable with oral folic acid supplemention *(24,25)*, suggesting that this metabolic block can be overcome with pharmacological doses of folic acid.

This heat-labile variant of MTHFR has been shown to result from a cytosine-to-thymine (C-to-T) mutation at nucleotide 677 *(26,27)*. Homozygotes for the TT genotype have higher tHcy levels compared with heterozygotes CT and normal homozygotes CC *(28)*. This is most pronounced in homozygotes with low serum folate levels. A second common polymorphism in the MTHFR gene (*A1298C*) is a glutamic acid-to-alanine substitution at codon 429 *(29,30)*, which also results in decreased MTHFR activity. An estimated 10% of the Canadian and Dutch populations *(29,30)* are homozygous (CC) for this polymorphism. The combined MTHFR *677TT* and *1298CC* genotypes are more rare than expected in the general population, which raises the possibility of a phenotype that is selected against during development *(31,32)*. Although individuals homozygous for the *A1298C* polymorphism do not have increased homocysteine levels or lower plasma folate, individuals who are heterozygous for both polymorphisms (approx 20% of a Caucasian population) have reduced MTHFR activity, higher homocysteine, and reduced plasma folate, similarly to individuals who are homozygous for the *C667T* polymorphism *(29)*.

2.3. Total Homocysteine Concentration in the Population

The concentration of tHcy in plasma among healthy, disease-free individuals ranges between 5 and 15 μmol/L *(8)*. Figure 2 shows mean levels of tHcy for male and female adults by age group based on National Health and Nutrition Examination Survey (NHANES) III data *(33)*. The 95% confidence intervals (CIs) are shown for two 4-yr age groups (12–15 yr and 16–19 yr) and six 10-yr age groups ranging from ages 20–29 yr to 80+ yr.

Plasma tHcy is dependent on age and gender, with plasma homocysteine concentrations increasing with increasing age. As shown in Fig. 2, and as is generally found in population-based studies, men have higher tHcy concentrations than women *(17,34)*. In women, homocysteine levels are possibly related to menopausal status *(10)*. On the other hand, large differences between pre- and postmenopausal women found when measuring homocysteine-cysteine mixed disulfide *(6,35)* were not found when measuring tHcy.

The reasons for the higher tHcy concentrations observed in older subjects may be secondary to decreased enzyme activity, depressed renal function, and other changes associated with aging. Gartler et al. *(36)* showed that cystathionine β-synthase activity in healthy subjects decreased with age; therefore, persons older than age 70 yr and particularly the very elderly had very low enzyme activity. Bostom et al. *(37)* used a rat model to document that loss of homocysteine metabolizing capacity of the kidneys may be a determinant of elevated tHcy concentration. A decreased intake and absorption of the vitamin cofactors responsible for homocysteine metabolism might also contribute. The consistent results of higher tHcy levels in the older population generally support these findings.

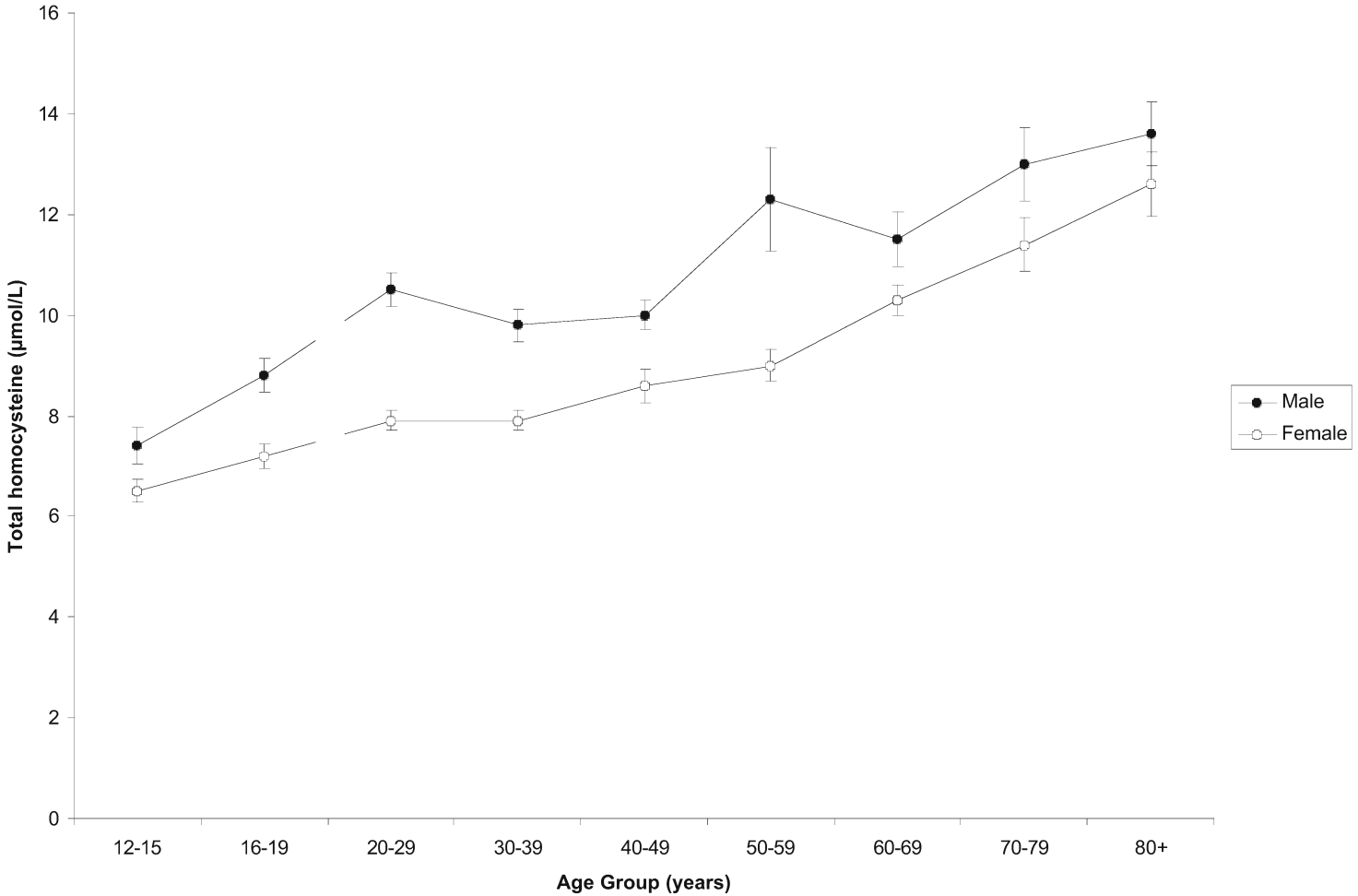

Fig. 2. Mean levels of plasma homocysteine in males and females (from NHANES III) *(31)*.

3. FOLATE AND HOMOCYSTEINE

3.1. The Influence of Folate Intake on Plasma Homocysteine

Several investigators have conducted studies examining the effect on homocysteine levels in response to changes in folic acid intake. Many of these studies were included in a careful meta-analysis published by the homocysteine lowering trialists' collaboration, which was guided by the Clinical Trial Service Unit in Oxford, UK *(38)*. The meta-analysis used individual-level data contributed by the collaborating authors. The results confirmed that folic acid supplements decrease homocysteine levels at all levels of homocysteine but that the amount of reduction was greater for higher baseline levels and lower blood folate levels, as had been suggested previously *(39,40)*. At average pretreatment values of homocysteine (12 μmol/L) and folate (12 nmol/L), added folic acid of at least 0.5 mg/d reduced homocysteine levels by about 25% *(38)*. The addition of vitamin B_{12} to folic acid supplements further reduced homocysteine levels by about 7%. Supplementation with vitamin B_6 had little additional effect on homocysteine levels. (The meta-analysis did not include an evaluation of PML levels of homocysteine.)

We are aware of several additional studies, not included in the meta-analysis, of healthy subjects with normal concentrations of folate, vitamin B_{12}, and vitamin B_6, in whom the effect of added folic acid alone for at least 3 wk could be evaluated. These findings are summarized in Table 1. Two studies were conducted in the United States before 1998 *(41,42)*, four were conducted in the United Kingdom *(43–46)*, three were conducted in The Netherlands *(47–49)*, one was conducted in Germany *(50)*, one was conducted in Finland *(51)* and one was conducted in Ireland *(52)*. In all these studies, there was no fortification with folate at the time.

These studies, conducted in populations without the exposure of increased folic acid in enriched flour and grain products, suggest a graded benefit at doses of folic acid below 0.5 mg/d. If there is indeed a graded benefit, it may not extend above about 0.5 mg of folic acid daily, because the meta-analysis *(38)* suggests that a plateau may be reached at about 500 μg/d of added folic acid. This existence of a plateau is supported by the result of Ubbink and colleagues *(53)* in which subjects, after 6 wk of treatment with 1000 μg of folic acid, 0.4 mg of cobalamin, and 12.2 mg of pyridoxine, had no additional lowering of tHcy when twice these amounts were given. The recent randomized trial by Oort and colleagues *(49)* also supported the existence of a plateau. This study used seven doses of folic acid (including placebo) to characterize the dose–response curve and concluded that about 90% of the maximal reduction of homocysteine is achieved with the 400 μg/d dose of folic acid.

The studies in Table 1 showed that supplemental folic acid decreases tHcy, and four of the studies also investigated the effects of natural food folate on tHcy or included folic acid in breakfast cereals *(48,51,54,55)*. Most of the dietary folate groups evaluated experienced significant increases in plasma folate and red blood cell folate concentrations, as well as significant reductions in fasting plasma tHcy. Although the amount of folate consumed by the dietary folate groups reported in Table 1 is higher than commonly consumed in the general population, these studies show that folate-dense foods can significantly enhance folate status and decrease tHcy concentration. These results are consistent with the studies of folic acid supplements.

Two randomized studies have been conducted in the United States during the post-fortification period *(56,57)*. In a small depletion-repletion study of 30 elderly women

Table 1
Homocysteine Response to Folic Acid

Author	*Country*	*Number*	*Gender*	*Dose of folic acid*	*Reduction in tHcy*	*Comments*
Homocysteine Lowering Trialists Collaboration, 1999	Various (none had fortification at the time of the study)	1114	M/F	400–5000 µg	0.8–11.6 µmol/L	Meta-analysis of trials of both healthy individuals and those with elevated tHcy. Minimum treatment period of 3 wk.
Trials on healthy populations not included in the Homocysteine Lowering Trialists' meta-analysis[a]						
In populations not exposed to fortification with folic acid						
O'Keefe et al., 1995 *(41)*	United States	17	F	170 µg 270 µg 370 µg	Comparison 2.7 µmol/L 4.2 µmo/L	Depletion then supplementation. Only final tHcy values were available, so relative reductions have been calculated.
Ward et al., 1997 *(46)*	Northern Ireland	30	M	100 µg 200 µg 400 µg	0.8 µmol/L 1.5 µmol/L 1.8 µmol/L	Doses were administered sequentially. Supplement users were excluded. Treatment periods were 6, 6, and 14 wk, respectively.
Jacob et al., 1998 *(42)*	United States	8	F	55 µg	0.0 µmol/L	Depletion-repletion; 3-wk treatment period.
Nelen et al. 1998 *(47)*	Netherlands	49	F	500 µg	3.6 µmol/L	No comparison group: before-after comparisons; 8-wk treat ment period; participants had history of recurrent spontaneous miscarriage.
Brönstrup et al., 1998 *(50)*	Germany	51	F	400 µg	0.95 µmol/L	Reduction compared to placebo washout; 4-wk treatment periods.
Brouwer et al., 1999 *(48)*	Netherlands	144	F	250 µg 500 µg	1.3 µmol/L 2.6 µmol/L	Supplement users were excluded; 4-wk treatment period.

Brouwer et al., 1999 *(176)*	Netherlands	67	M/F	350 μg of dietary folate 250 μg of folic acid	2.1 μmol/L 2.4 μmol/L	Folate levels are additional to those of the placebo group; 4-wk treatment period.
Riddell et al., 2000 *(54)*	New Zealand	65	M/F	470 μg of dietary folate 300 μg of folic acid in breakfast cereal 440 μg of folic acid	0.4 μmol/L 2.4 μmol/L 2.2 μmol/L	Comparisons with control group; 12-wk treatment period.
Silaste et al., 2001 *(51)*	Finland	37	F	380 μg of dietary folate	1.1 μmol/L	Crossover trial: 5-wk treatment periods with 3-wk washout. Difference in dietary folate equivalent to 223 μg of folic acid.
Daly et al., 2002 *(52)*	Ireland	95	F	100 μg 200 μg 400 μg	0.16 μmol/L 0.41 μmol/L 1.21 μmol/L	Ten-week treatment period. Changes in relation to placebo group calculated.
Venn et al., 2002 *(55)*	New Zealand	70	M/F	100 μg in fortified 200 μg breakfast 300 μg cereal	1.8 μmol/L 1.3 μmol/L 2.1 μmol/L	Four-week treatment period. Differential change calculated in reference to the control group.
Rydlewicz et al., 2002 *(43)*	United Kingdom (Scotland)	368	M/F	50 μg 100 μg 200 μg 400 μg 600 μg	1.34 μmol/L 0.54 μmol/L 1.41 μmol/L 1.98 μmol/L 1.87 μmol/L	Six-week treatment period. Differential change calculated in reference to the placebo group after 3-wk run-in.
Ashfield-Watt et al., 2002 *(44)*	United Kingdom (Wales)	126	M/F	246 μg in fortified foods 352 μg in supplement	2.2 μmol/L 2.3 μmol/L	Sixteen-week treatment periods; crossover trial. Changes relative to exclusion diet period. Dose takes account of compliance (88%)
Ashfield-Watt et al., 2003 *(45)*	United Kingdom (Wales)	135	M/F	100 μg natural folate 100 μg folic acid	0.61 μmol/L 0.47 μmol/L	Sixteen-week intervention periods. All participants had wild-type CC genotype for MTHFR *C677T*.

(Continued)

Table 1 *(Continued)*

Author	*Country*	*Number*	*Gender*	*Dose of folic acid*	*Reduction in tHcy*	*Comments*
Van Oort et al., 2003 *(49)*	Netherlands	316	M/F	50 µg 100 µg 200 µg 400 µg 600 µg 800 µg	0.8 µmol/L 1.3 µmol/L 2.2 µmol/L 2.8 µmol/L 3.4 µmol/L 2.8 µmol/L	Differential change calculated in reference to placebo. Twelve-week treatment period. Stratification by quartile of tHcy before randomization.
In populations exposed to fortification with folic acid						
Kauwell et al., 2000 *(56)*	United States	30	F	10 µg of folic acid + 70 µg dietary folate 86 µg of folic acid 137 µg of folic acid + 155 µg of dietary folate 311 µg of folic acid	Comparison 0.9 µmol/L 1.3 µmol/L 2.8 µmol/L	Depletion (to 110 µg of folate)-repletion. Results in comparison to low folate group. Folate values are added to 110 µg of dietary folate; 7-wk treatment periods; elderly women.
Beresford et al., 2004 *(57)*	United States	160	M F	200 µg 400 µg	0.4 µmol/L 0.4 µmol/L	Two crossover trials with two 12-wk treatment periods, 30 wk of washout period between treatments. Stratification by MTHFR genotype, gender, and broad tHcy level before randomization.

[a]Restricted to studies or parts of studies with at least 3 wk of intervention.

(56), participants followed a low-folate diet (110 µg of dietary folate) for 7 wk and were randomized to one of four groups with varying combinations of orange juice or folic acid provided in apple juice. Significant additional lowering of homocysteine levels occurred in response to the equivalent of 300 µg additional folic acid *(56)*. In a study of 160 volunteers (men and women), in which persons homozygous for the TT genotype of MTHFR C677T were overrecruited, a dose of 200 µg of folic acid given for 12 wk was compared to a placebo. The design was a crossover trial with a long washout period (30 wk), so that each person served as his or her own control. The order of treatments was randomized. A similar parallel trial compared the dose of 200 µg with 400 µg of folic acid. The estimates of additional lowering of tHcy beyond placebo were significant for both doses *(57)*. Taken together, these studies suggest that the plateau of lowering of homocysteine attributable to folic acid has not yet been achieved with current levels of fortification.

3.2. Homocysteine and Folate Concentrations

The lowering of homocysteine levels in response to increasing folate intake is further supported by the inverse relationship between blood folate and tHcy. Seven studies *(8,10,58–62)* of tHcy and folate found that tHcy levels markedly increased as plasma/serum folate levels decreased. Folate levels were determined from blood *(10)*, plasma *(59,60)*, or serum *(8,58)* samples and quantified by radioassay *(58,62)* or microbiological assay *(59,60)*. Five additional studies *(14,34,63–65)* provided support for a strong inverse association between tHcy levels and plasma/serum folate levels.

Kang et al. *(58)* measured serum protein-bound homocysteine in 239 stored samples that represented the extremes of depletion or sufficiency of folate or vitamin B_{12} among 1826 subjects seen in a 1-yr period at a major metropolitan medical center. The mean age of included subjects was 63.7 yr (range: 16–97 yr), with 56% of subjects being female. Among subjects with subnormal serum folate <4.5 nmol/L), 84% had homocysteine values that exceeded two standard deviations (SDs) above the mean for a normal group. Homocysteine levels did not appear to change significantly above 9.1 nmol/L. Because carefully conducted assessment of dietary folate intake reflects biochemically measured folate status *(66)*, these findings are consistent with an inverse association between tHcy levels and dietary folate over the low portion of the range of folate intake, with a flattening out of the relationship at the high end, as has been suggested by the studies of folate intake. The criteria used to evaluate plasma folate levels in the absence of vitamin B_{12} deficiency are: persons with levels less than 6.7 nmol/L are at "high risk," persons with levels between 6.7 and 11.1 nmol/L are at "moderate risk," and persons with levels greater than 11.1 nmol/L are at "low risk" *(66)*.

Since the introduction of fortification of enriched flour with folic acid, studies in the United States have found substantial changes in the distribution of both tHcy and plasma folate. One study was particularly informative because of information from an ongoing cohort. As part of the regular follow-up examinations of the Framingham offspring cohort, fasting blood samples were obtained, and all participants completed a food-frequency questionnaire containing information on vitamin supplements. The timing of the fifth examination was such that it was completed before fortification started. For a portion of the cohort, the sixth examination also occurred before fortification, whereas for another portion, the exam occurred after fortification. Members of the cohort whose sixth examination occurred between October 1996 and August 1997 were excluded from the

analysis because fortification had begun but was not yet fully implemented *(67)*. The increase in plasma folate attributable to fortification was about 5 ng/mL. Among the subgroups of the cohorts not taking B-vitamin supplements, the reduction in tHcy attributable to fortification was about 0.9 µmol/L. Interestingly, although the sixth examination tHcy levels in the subgroups taking B-vitamin supplements were lower on average than in the rest of the cohort, the change in tHcy attributable to fortification was an increase of 0.5 µmol/L. Of note, the difference in the proportion of persons with tHcy greater than 13 µmol/L postfortification was not significant after adjusting for B_6 and B_{12} differences between users and nonusers of B-vitamin supplements.

3.3. Pyridoxine and Vitamin B_{12}

Cobalamin alone is effective in lowering tHcy levels in cases with overt cobalamin (vitamin B_{12}) deficiency *(28,68–70)*. In 20 subjects with vascular disease with elevated tHcy values *(68)*, treatment with pyridoxine alone improved the result of the methionine loading test, whereas the inclusion of folic acid resulted in a marked decrease in basal tHcy levels, especially in subjects with hyperhomocysteinemia. From this and other studies *(71)*, it appears that pyridoxine may be effective in reducing elevated tHcy following a methionine load test but is not effective in reducing the elevated fasting tHcy. Dudman et al. *(72)* found that treatment with either pyridoxine or folic acid was similarly effective to those from combined treatment for subjects with vascular disease as assessed with a PML test. On the other hand, folic acid seems to be the key factor in reducing fasting hyperhomocysteinemia.

4. HOMOCYSTEINE AND CVD

4.1. Homocysteine and CHD

Many studies have been conducted concerning various kinds of vascular diseases and their relationship to tHcy levels and/or folate levels or folate intake. Study designs varied from prospective to cross-sectional to case-control studies, in which controls were selected from a source population different than that of the cases. These source populations of controls ranged from volunteers to hospital patients. Other studies recruited controls from screening programs or population-based registries. Some studies included cases of both fatal and nonfatal myocardial infarction (MI), whereas others included only nonfatal cases, sometimes defined by degree of angiographically confirmed occlusion of a coronary vessel. Some studies measured PML levels, whereas others used fasting or basal (casual) levels of homocysteine. Basal levels are easier to obtain than fasting levels in large cohort investigations. Our group has summarized, in detail, studies identified through 1999 *(73–75)*, and overlapping sets of studies have been subjected to critical review or meta-analysis by other groups *(76–83)*. The meta-analyses reporting pooled risk of CHD are summarized in Table 2.

Nested case-control studies are those in which controls were sampled from the cohort from which the cases arose; blood samples were obtained (and frozen) before the disease occurred. Population-based case-control studies are retrospective studies in which cases and controls were drawn from the same defined general population. These two study types are considered to be “high quality” but were separately synthesized in most meta-analyses. These analyses differed in their treatment of cross-sectional and “other” case–control studies. Cross-sectional studies used no sampling by outcome status and

Table 2
Meta-Analyses of Homocysteine and CHD Risk Estimated Odds Ratio (95% Confidence Interval)

Author	*Unit of tHcy change used in odds ratio*	*CHD prospective*	*CHD retrospective*	*Number of studies*
Studies that did not differentiate population-based case-control studies from other case-control studies				
Cleophas et al., 2000 *(78)*	"Elevated" tHcy	1.49 (1.33–1.67)	1.62 (1.50–1.74)[a]	11 prospective 22 retrospective
Ford et al. IJE, 2003 *(82)*	5-µmol/L change	1.23 (1.14–1.32)[b]	1.70 (1.50–1.93)	10 prospective 29 retrospective
Studies that only included population-based case-control studies in the pooled estimate of retrospective studies				
Ueland et al., 2000 *(77)*	5-µmol/L change	1.20 (1.14–1.25)	Not included	14 prospective
Beresford and Boushey, 2001 *(73)*	5-µmol/L change	1.15 (1.09–1.21) male	1.35 (1.24–1.48) male	12 male, 7 female prospective
		1.42 (1.25–1.62) female	1.37 (1.15–1.55) female	6 male, 5 female population-based case-control
The Homocysteine studies collaboration, 2002 *(79)*	25% lower baseline homocysteine level calculated inverse	0.83 (0.77–0.89) 1.20 (1.12, 1.30)	0.67 (0.62–0.71) 1.49 (1.41, 1.61)	11 prospective 12 population -based case-control
Bautista et al., 2002 *(81)*	"Elevated" tHcy	1.46 (1.15–1.86)	Not included	9 prospective only
Wald et al., 2002 *(83)*	5-µmol/L change	1.23 (1.14–1.32)	Not included	16 prospective only

[a]Reported as CAD but actually included in some studies of cerebrovascular disease.
[b]Only nested case–control studies included in this pooled estimate.
CHD, coronary heart disease; CAD, coronary artery disease.

may have included convenience samples. Other case–control studies included hospital-based investigations and those in which controls were from a different population than the cases. Some researchers decided against conducting a formal meta-analysis of these studies because of the variability in study methods and quality *(76)*. Others assigned quality scores to each study based on design criteria *(82)*.

There is still no consensus on the exact level for defining elevated tHcy. The definitions commonly employed are values exceeding the 90th or 95th percentile among a group of healthy subjects or values exceeding the mean of normal controls by more than two SDs. Therefore, the cutoff values used by investigators for classification of elevated tHcy levels in their respective studies vary according to the distribution among their control subjects. This presents a challenge for summarizing the evidence concerning the link between hyperhomocysteinemia and risk of CHD in a quantitative fashion.

Although early studies examined the risk of CVD associated with elevated homocysteine, many later studies found evidence of a graded relationship between homocysteine and risk of cardiovascular disease *(84–88)*. Indeed, several studies *(89–91)* of carotid and coronary arteries indicated that the extent of quantitatively defined arterial narrowing is consistent with a graded response to increasing tHcy levels. As described earlier, pooling information from studies that used disparate definitions of elevated homocysteine leads to grouping of quite disparate estimates of risk. Nonetheless, this approach was used in a few of the meta-analyses *(78,81)*. Studies using cut-points for elevated tHcy also can contribute to estimates of the linear association between tHcy and CHD risk.

Analyses from our group *(73–75)*, as well as some others *(82,83)*, assumed a linear relationship between tHcy levels and risk of CAD and calculated a summary odds ratio based on a 5-µmol/L increment in tHcy concentration. The combined odds ratio for a 5-µmol/L increment in tHcy, adjusting for conventional risk factors for CHD as reported in the individual studies, is shown in Table 2. It should be noted that some prospective studies included in these meta-analyses reported being able to distinguish between short-term and longer term risk of CHD associated with high homocysteine (e.g., refs. *92–94*). However, the meta-analysis of prospective studies by Bautista and colleagues *(81)* found that the magnitude of the combined risk of all CVD was independent of the duration of follow-up. Again, some of the studies found evidence of an interaction effect *(77)* with one or more of the conventional risk factors *(15)*. On the other hand, this was not confirmed in the large collaborative study of British, European, and American scientists in which some of our group participated, known as the Homocysteine Studies Collaboration *(79)*. This collaboration obtained primary data from 30 studies, allowing a powerful set of analyses that examined differences between studies, confounding factors, and regression dilution bias.

There was no evidence of interaction between the association of homocysteine with CHD and age, gender, smoking, blood pressure, or blood cholesterol *(79)*. Homocysteine was correlated with the major known risk factors for CHD, namely current smoking, total cholesterol, and systolic blood pressure, and adjustment for these factors did attenuate the size of the association between tHcy and risk of CHD. These risk estimates, calculated using a multiplicative model (using a logarithmic transformation of tHcy), are presented in Table 2. The estimates relate to a 25% lower than baseline shift in tHcy, which is equivalent to about 3 µmol/L at the mean tHcy in controls, 4 µmol/L at mean plus one SD, and 5 µmol/L at mean plus two standard deviations,. Because the other meta-analyses have calculated pooled risks in relation to elevated tHcy or tHcy of

5 µmol/L higher, we have included a recalculation of the published estimate and two ends of the CI, using the inverse of each number, for comparison purposes.

Several authors claimed that the relative risks estimated from prospective studies were less likely to show an effect of tHcy elevations on CHD than those estimated from case-control studies (including population-based). Although this has been documented in several studies that conducted careful meta-analyses separately for these two types of studies, it did not hold universally true, as demonstrated in Table 2. For example, Beresford and Boushey *(73)* found that the estimated risk per 5 µmol/L tHcy was closer to the null for the case–control studies than for the prospective studies for women. Overall, the pooled odds ratio for CHD for men and women together is consistently around 1.2 per 5 µmol/L tHcy change and around 1.5 for elevated tHcy.

4.2. Homocysteine and Cerebrovascular Disease

Studies of homocysteine and risk of stroke also have been summarized by our group *(74,75)* as well as others *(79,80,82,83)*, and these summaries have included both prospective studies and retrospective case-control studies. The various meta-analyses of homocysteine and risk of cerebrovascular disease are shown in Table 3. Four studies of presymptomatic carotid arteriosclerosis provide additional support for tHcy as a risk factor in cerebrovascular disease. Evidence of carotid atherosclerosis using ultrasound methods was noted in heterozygotes for cystathionine β-synthase deficiency *(95,96)*. Two larger cross-sectional studies also showed an association of hyperhomocysteinemia with objective evidence of carotid artery stenosis *(90,91)*. From prospective studies, the pooled odds ratio for stroke per 5 µmol/L tHcy change has been estimated at between 1.3 and 1.6, as shown in Table 3.

4.3. Homocysteine and Peripheral Vascular Disease

Three population-based case–control studies *(97–99)* were part of our earlier synthesis of homocysteine and peripheral vascular disease studies *(14,68,73,97–103)*, and two others have been published since 1996 *(15,104)*. All five studies found homocysteine to be a risk factor for peripheral vascular disease, independent of conventional risk factors. The Framingham study *(105)*, with a median follow-up of 10 yr, also found an increased risk of CVD (including peripheral vascular disease) of 1.53 (95% CI 1.16, 1.98), associated with homocysteine levels in the highest quartile compared to the other three quartiles after adjustment for age, gender, cholesterol (total and HDL), smoking, blood pressure, and diabetes.

5. MTHFR, FOLATE INTAKE, AND CVD RISK

Given the evidence that homocysteine is related to increased risk of CVD and that individuals homozygous for MTHFR *C677T* have decreased MTHFR activity and higher homocysteine levels, it seems plausible that individuals with the TT mutation might experience an increased risk of MI. The hypothesis is that high folate intake might compensate the lower MTHFR activity in homozygotes by lowering plasma tHcy in individuals with the mutation. One of the first studies to examine this hypothesis was the Physicians' Health Study *(106)*. During an 8-yr follow-up, the risk for nonfatal MI and fatal CHD was not significantly different between the three *C677T* genotypes, despite the TT genotype having significantly higher mean tHcy levels than the CC

Table 3
Meta-Analyses of Homocysteine and Stroke Risk Estimated Odds Ratio (95% confidence interval)

Author	*Unit of tHcy change used in odds ratio*	*Stroke prospective*	*Stroke retrospective with population controls*	*Number of studies*
Studies that did not differentiate population-based case-control studies from other case-control studies				
Kelly et al., 2002 *(80)*	"Elevated" tHcy	1.43 (1.09–1.89) HR 2.01 (0.97–4.13) OR	1.79 (1.61–2.00)[a]	6 prospective (3 with HR, 3 with 14 combined prospective and retrospective
Ford et al., 2002 *(82)*	5-µmol/L change	1.58 (1.35–1.85)	2.16 (1.65–2.82)	6 nested case-control 20 retrospective
Studies that only included population-based case-control studies in the pooled estimate of retrospective studies				
Beresford and Boushey, 1997 *(74)*	5-µmol/L change	1.5 (1.3–1.8)[a]	Not included	3 prospective combined with 1 population-based case-control
The Homocysteine studies collaboration, 2002 *(79)*	25% *lower* homocysteine level	0.77 (0.66–0.90)	0.86 (0.73–1.01)	8 prospective
	Calculated inverse	1.30 (1.11, 1.52)	1.16 (0.99, 1.37)	3 population-based case-control
Wald et al., 2002 *(83)*	5-µmol/L change	1.42 (1.21–1.66)	Not included	8 prospective only

[a]Combined all studies. HR, hazard ratio; OR, odds ratio.

genotype. This difference was most marked among men with low folate levels. Thus, in this population, the MTHFR *C677T* polymorphism was associated with higher tHcy levels but not with risk of MI *(106)*. Many other studies addressing the role of the MTHFR polymorphism and its effect of vascular disease *(107,108)* reported similar results. More recently, a family-based study in Spain *(109)* used the transmission disequilibrium test to evaluate the associations of the MTHFR *C677T* gene variants and CHD risk. In this study, 101 nuclear families (parents) were recruited from index cases. No difference in T-allele frequency was found, even within subgroups defined by major CHD risk factors. One meta-analysis of 23 reports in the literature *(28)* concluded the prevalence of the TT genotype in patients with CVD was almost identical to the prevalence in controls (11.9 and 11.7% respectively). A meta-analysis of stroke risk reported the pooled odds ratio from 19 studies associated with the TT genotype as 1.23 (95% CI 0.96–1.58) *(80)*. On the other hand, two recent meta-analyses did find a significant elevated risk associated with the TT genotype. In a meta-analysis of 46 studies of CHD in relation to TT genotype, the pooled odds ratio for homozygotes for *677TT* compared with wild-type *CC677* was 1.21 (95% CI 1.06–1.39) *(83)*. Similarly, a meta-analysis that included 40 studies (22 from Europe, 10 from North America, and 8 from other continents) *(110)* found an overall odds ratio of 1.16 (95% CI 1.05–1.28). There was heterogeneity between studies that was largely accounted for by continent of origin. The pooled risk of CHD among individuals with TT genotype compared to those with CC genotype within the European studies was 1.14 (95% CI 1.01–1.28), whereas the corresponding risk within North American studies was 0.87 (95% CI 0.73–1.05). This difference may be explained by differences in folate status, as suggested by Klerk and colleagues *(110)*. A subset of the studies included data on folate status, so that the effect of TT genotype on CHD risk could be estimated separately by folate status. There was a significant elevation among those with low status, who had a pooled odds ratio of 1.44 (95% CI 1.12–1.83), but not among those with high folate status, who had a pooled odds ratio of 0.99 (95% CI 0.77–1.29) *(110)*. Therefore, most of the evidence suggests that there is an elevated risk of CHD associated with TT homozygotes for the MTHFR *C677T* polymorphism *(111)*; this association has been difficult to prove because of the small overall effect but is apparent among those with concomitant low folate status. This evidence of the role of MTHFR *C677T* is consistent with the relationship between homocysteine and CHD risk.

6. FOLATE AND CVD

6.1. Dietary Folate and Folate Concentration and Risk of CVD

Many studies of CVD and tHcy levels also measured the B-vitamins that most influence tHcy concentration, namely, folate, vitamin B_6, and vitamin B_{12}. In particular, folate is strongly inversely associated with levels of tHcy, and if tHcy is associated with CVD, then of the link between folate status and risk of CVD is important. The findings among these observational studies have been reviewed previously *(73,74)*. In spite of different definitions of case and the use of different cut-points of the folate distribution, the results of the studies are fairly consistent. At concentrations of plasma/serum folate above 10 nmol/L, there is a significant reduction in risk for vascular disease *(89,91,112,113)*, particularly among females *(108,114)* and adults ages 35 to 55 yr *(115)*. Point estimates from other studies *(93,116,117)* supported these findings but were not statistically significant.

In the studies that had available levels of tHcy as well as concentration of folate, investigators were able to examine the association of folate with vascular disease while simultaneously controlling for fasting tHcy. Theoretically, if the risk associated with folate and vascular disease changes with the addition of tHcy in the model, then one can assume that folate is affecting the risk of vascular disease via the tHcy pathway. In the population-based case–control studies reported by Pancharuniti et al. *(89)* and Robinson et al. *(113)*, the addition of tHcy to their models of folate and CVD resulted in folate no longer being significant. In the study by Schwartz et al. *(116)* among younger aged women, the association with folate was weakened when tHcy was added to the model. In the prospective Physicians' Health Study, the presence of tHcy in the model only slightly attenuated the RR for low levels of folate; therefore, it was not clear in this study whether folate affected the risk of MI only via the tHcy pathway or had an independent effect.

Regardless of the pathway, there is enough evidence to justify a targeted research effort to explore the relationship between intake of dietary folate and risk for vascular disease. Early studies used observational data to examine this question. In a subset of subjects from the Framingham Heart Study *(91)*, stenosis was inversely related to reported intakes of folate. Intakes less than 327 μg/d showed a fivefold increase in risk compared to intakes greater than 475 μg/d. Another analysis from the Framingham Study *(118)* indicated that intake of fruits and vegetables was inversely associated with incidence of strokes and transient ischemic attacks (TIAs). Although many factors could potentially explain this finding, one consideration is the large contribution that fruits and vegetables made to total folate intake. Therefore, the protective effect of fruits and vegetables for stroke and TIA is compatible with folate lowering the risk of CVD, possibly through lowering tHcy levels. In a careful analysis of the NHANES I Epidemiologic Follow-Up Study, dietary intake of folate was assessed using information from the single 24-h dietary recall (at baseline in 1971–1975), and information about folate content of foods reported was calculated using ESHA Food Procesessor software *(119)*. At about 19 yr of follow-up (in 1992), 3758 incident cardiovascular events had occurred, of which 926 were incident stroke events *(120)*. The median dietary folate intake in the early 1970s was approx 200 μg/d. Analyses adjusted for energy intake and known major risk factors for stroke and CVD. Participants who consumed at least 300 μg/d had a 20% lower risk of stroke and a 13% lower risk of any CVD. Limitations of the single 24-h recall were acknowledged, but within-person variability would most likely lead to underestimation of the association between dietary folate and stroke risk *(120)*.

A small case–control study of dietary folate intake and risk of nonfatal MI was conducted in Spain. Compared to the lowest quartile of energy-adjusted folate intake (estimated from a food-frequency questionnaire), the upper three quartiles had an odds ratio of 0.57 (95% CI 0.35–0.94) *(121)*. Graham et al. *(15)* found that users of vitamin supplements had a substantially lower risk of vascular disease than nonusers, and some of this lowering was attributable to lower plasma tHcy levels. A prospective study in Finland based in Kuopio, which has one of the highest rates of CHD, included a 4-d food record at baseline (1984–1989). By the 10-yr follow-up, 199 men had experienced an acute coronary event. After adjusting for major known risk factors and other conventional and nutritional factors, men in the highest quintile of folate intake at baseline (300 μg/d or more) had a relative risk of acute coronary events of 0.45 (95% CI 0.25–0.81) compared to those in the lowest quintile of folate intake (about 200 μg/d or less) *(122)*.

Additional evidence for dietary and supplement sources of folate comes from the prospective analysis of the Nurses' Health Study by Rimm et al. *(123)*. During a 14-yr follow-up, the risk for nonfatal MI and fatal CHD was considerably lower among women who consumed 696 µg of folate per day compared to 158 µg (RR = 0.69, 95% CI 0.55–0.87). Risk of CHD was also reduced among women who regularly used multiple vitamins (the major source of folate), including vitamin B_{12} and B_6 (RR = 0.76, 96% CI 0.65–0.90).

Several randomized controlled trials of folate, vitamin B_{12}, and vitamin B_6 are being conducted or have concluded recently. One small study of an intervention to reduce carotid plaque area provided evidence of vitamin supplementation influencing the progression of atherosclerosis *(124)*. This study followed 18 men and 20 women (mean age: 57.9 yr) with initial tHcy concentration greater than 14 µmol/L for 4.4 yr after treatment with 2.5 mg of folic acid, 25 mg of pyridoxine, and 250 µg of vitamin B_{12}. Each individual had at least two measurements of total plaque area before and two measurements after treatment as determined by ultrasound. Prior to treatment, plaque area had been measured as increasing. After treatment, plaque area decreased significantly and both genders and all ages responded equally to treatment with the B vitamins *(124)*. No concentration levels of vitamins or tHcy were reported. Two slightly larger trials were recently published *(125,126)*. In a randomized controlled study of 5000 µg/d of folic acid, 593 patients in the Netherlands with stable CHD and currently on statin treatment were randomized to intervention or control and followed for approx 2 yr. A fortification program in the Netherlands had not been implemented at the time of the study. Nonetheless, the study found no difference in time to first event of any of a variety of CVD events for the folic acid-supplemented group compared to the placebo group *(125)*. In contrast, the randomized controlled trial in Switzerland of 1000 µg/d of folic acid with 400 µg/d of vitamin B_{12} and 10 mg/d of vitamin B_6 found a lower risk of death, nonfatal MI, and need for repeat revascularization at 1 yr of a composite endpoint *(127)*. Participants were 553 consecutive patients enrolled after successful angioplasty of at least one significant coronary stenosis who were not taking vitamin supplements. The treatment period was 6 mo. Clinical follow-up took place at 6 mo and again at 12 mo postrandomization. The hazard ratio of any event was 0.66 (95% CI 0.47–0.94) in the folic acid, B_{12}, and B_6-treated group compared to the placebo after adjustment for factors known to influence the need for target lesion revascularization *(127)*. None of these trials was large, and all had relatively short-term follow-up.

Studies in countries such as the United States and Canada, where fortification of enriched flour and grain products has already been implemented, may need even larger numbers because the reduction in levels of homocysteine with additional folic acid, B_{12}, and B_6 treatment is likely smaller than would have been expected prior to fortification (*see* Table 1). It has been pointed out that useful inferences may still be made from studying subsets of persons with very high levels of homocysteine, such as those with renal impairment *(128)*, because renal function is highly correlated with homocysteine levels, especially in those over age 65 yr *(129)*. One large study was recently published *(130)*. Participants were individuals who had a nondisabling stroke and total homocysteine levels in the top quartile of the North American homocysteine distribution. Centers collaborated from the United States, Canada, and Scotland. After a run-in period, 3680 participants were randomized to high dose or low dose of folic acid, pyridoxine, and cobalamin. The study was stopped early, after an average of 20 mo, with

essentially no difference in the risk of recurrent stroke *(130)*. The reduction in follow-up homocysteine levels in the group assigned to receive high doses of vitamins was not as large as had been anticipated, and even this study was insufficiently powered to detect the corresponding difference (predicted from observational studies) in combined CHD and stroke risk.

There are at least nine other large randomized trials underway for which results currently are not known. The minimum sample size projected is 2000, and the maximum is 12,000 *(131,132)*. There are two studies in Norway (NORVIT, WENBIT), two in Australia (PACIFIC *[133]*, VITATOPS), one in the United States (WACS), one in Canada (HOPE-2), two in the United Kingdom (SEARCH, CHAOS-2), and one in France (SU.FOL.OM3 *[134]*). The interventions include various combinations of folic acid (at doses between 2000 and 5000 μg), vitamin B_{12} (at doses between 400 and 1000 μg), and vitamin B_6 (at doses between 25 and 50 mg). All are secondary prevention trials of stroke, CHD, or other vascular disease *(131)*.

7. SUMMARY AND CONCLUSIONS

7.1. A Causal Relationship Between Homocysteine and CHD

The evidence linking homocysteine levels with risk of CVD is consistent. Both prospective and case-control studies suggest a positive association. Although there is some evidence of confounding, there is also evidence regarding CHD that tHcy is an independent risk factor, contributing additional risk independent of some other known major risk factors such as total cholesterol *(89,92,135–138)*, LDL or HDL cholesterol *(89,92,138,139)*, body mass index *(89,92)*, age *(89)*, and high blood pressure *(89,92,137,138)*.

Although the magnitude of the association between tHcy and risk of CHD is not large, it has been estimated with good precision. In the various meta-analyses reviewed, the summary risk estimate for CAD for an increase of 5 μmol/L of homocysteine was about 1.2 after adjusting for conventional risk factors, using only the prospective studies. This level of risk is similar to other well-established risk factors for CHD.

There is no evidence that CAD or related diagnostic manipulations influence tHcy concentrations. In the prospective studies, high tHcy levels clearly antedated the manifestations of either CAD or stroke.

There are biologically plausible mechanisms by which tHcy might alter risk of developing vascular disease. Atherogenic mechanisms promoted by homocysteine include endothelial cell desquamation, oxidation of LDL, and monocyte adhesion to the vessel wall *(22,140)*. Direct toxicity of homocysteine to the endothelium has been reported in laboratory studies *(141–143)*. Harker et al. showed endothelial desquamation in vivo in baboons infused with homocystine *(144)* or homocysteine *(145)* at the high levels typical of patients with homocystinuria. Tsai and coworkers *(146)* showed that homocysteine increases DNA synthesis in vascular smooth muscle cells consistent with early arteriosclerotic lesions and induces these cells to proliferate while impeding the regeneration of endothelial cells. Homocysteine may predispose to arteriosclerosis by promoting oxidation of LDL *(147)* or by decreasing thrombomodulin cell-surface expression and inhibiting protein C activation, thus probably contributing to development of thrombosis *(148)*. Folate may have beneficial cardiovascular effects via endothelium-dependent responses, as was demonstrated in a crossover trial of adults with hyperhomocysteinemia *(149)*.

In summary, the association between hyperhomocysteinemia and CHD has the required elements of strength, consistency, temporality, and biological plausibility that allow a causal inference from observational studies *(150)*.

7.2. Limitations

In meta-analysis, results are combined from studies that may differ in methodology and include noncomparable populations. Publication bias (i.e., not publishing small negative studies) is possible. Although those negative studies that were published were included in the summary risk ratios, there may have been other negative studies performed for which lack of inclusion may have inflated the summary risk ratios calculated. The relationship between homocysteine and CHD risk is established from the observational studies, but to date, only a few short-term randomized controlled trials have been published that have investigated the effects of lowering of homocysteine on CVD markers *(151,152)*. More than nine large randomized controlled trials are now underway, and we await their results with interest.

Many investigations demonstrated that folic acid reduces tHcy levels. Although various combinations of folic acid, vitamin B_{12}, and vitamin B_6 were often administered, folic acid appeared to be the effective agent, because it reduced tHcy levels even when administered alone. Vitamin B_{12} contributes additional lowering of tHcy *(153,154)*. However, because the interactions of folic acid, vitamin B_{12}, and vitamin B_6 are not fully known, further studies of all three of these vitamins will be useful. Whether nutritional inadequacy of folic acid alone will raise tHcy levels or whether this response is limited to individuals predisposed genetically to develop hyperhomocysteinemia requires further study. The existence of several different genetic traits (including the common MTHFR polymorphism described earlier) predisposing to high tHcy levels suggests that genetic variability in response to folic acid intake might be expected. Therefore, it is not unlikely that TT homozygotes for the MTHFR polymorphism *(155)* may have higher folate requirements, and furthermore, it is possible that other subsets of the population may have different folate requirements (regarding homocysteine lowering capacity). Until such differences are fully elucidated, a population approach that treats the different genetic and environmental origins of the homocysteine–folic acid relationship in the same way appears appropriate.

7.3. The Broader Health Context

Are there negative implications of widespread supplementation or fortification with folic acid? It has been known for a long time that folic acid may mask the hematological manifestation of unrecognized pernicious anemia (cobalamin deficiency) while its neurological manifestations, which may be severe and include spinal cord damage, may progress *(156)*. However, such effects were observed mainly with high pharmacological doses of folic acid (i.e., 5,000 μg or more). It is not clear whether such high doses precipitate or exacerbate the neuropathy of cobalamin deficiency. The effect on the neuropathy of vitamin B_{12} deficiency of lower doses, such as those received through fortification of enriched flour and grain products in the United States, remains poorly defined *(156)*. Among the elderly, studies have estimated a 5% prevalence of serum vitamin B_{12} deficiency associated with methylmalonicacidemia *(62,70)*, unrelated to pernicious anemia. This deficiency could be corrected by oral doses of cobalamin, as has been demonstrated by some members of our group *(157)*. If the oral dose of vitamin B_{12}

were large enough (such as 1000 μg of B_{12}), even persons with pernicious anemia (estimated to be 1 in 5000) *(158)* would derive some benefit, because 1 to 3% of vitamin B_{12} can be absorbed by simple diffusion *(159,160)*. In countries that have implemented fortification with folic acid at low doses (140 μg/100 μg of grain product) as well as in those that have not, the highest consumers of folic acid continue to be those taking vitamin supplements containing 400 μg of folic acid. If 1000 μg of cobalamin was added to vitamin supplements containing 400 μg of folic acid, then most vitamin B_{12} deficiencies would be corrected, and concern about the possible masking effects of folic acid would likely be negligible *(156)*.

What are other implications of a nutritional policy to increase folic acid intake? About 2500 cases of neural tube defects occur annually in the United States, and about 1500 fetuses with this condition are aborted following detection by prenatal diagnosis *(161)*. Women who had children with neural tube defects have slightly elevated tHcy levels. In some women, this is caused by low folate intake or poor absorption, and in others, this is presumably caused by yet undescribed genetic defects or variants predisposing to hyperhomocysteinemia *(162)*. There is excellent evidence from a variety of studies that at least 50% of neural tube defects can be prevented if 400 μg of folic acid is taken daily before and during the first 4 wk of pregnancy *(161)*. Because many women do not realize it when they are in the very early stages of pregnancy, folic acid supplementation and dietary counseling have not been successful in increasing folic acid intake in the population during this critical time *(163)*. The Food and Drug Administration in the United States mandated adding folic acid to the food supply by fortification of enriched grains from 1998 onward *(164)*. The policy of fortification appears to have delivered more than the average population-wide increase of 100 μg/d that had been anticipated. A study of serum folate levels over time using stored bloods at Kaiser–Permamanente showed a 50% increase in median serum folate value between 1994 and 1998 *(165)*, and an application of a linear regression analysis using published studies of the relationship between folate intake and serum folate suggested a daily increase of about 200 μg in folate intake following fortification *(166)*. The fortification policy appears to have led to a reduction in the rate of neural tube defects of about 19% *(167)* and—in light of our review of the homocysteine, folic acid, and CVD risk story—at the same time promises to have the much larger effect of reducing vascular disease in several thousand older men and women.

8. RECOMMENDATIONS

Although the cause and mechanism of homocysteine associated CVD have not yet been firmly established, the American Heart Association deemed the association compelling enough to issue a Science Advisory in early 1999 *(168)*. Currently, widespread screening of tHcy levels is not recommended. However, determining fasting tHcy levels among affected individuals of younger age and in high-risk patients (i.e., individuals with a strong family history for premature atherosclerosis or occlusive vascular disease in the absence of other "traditional" risk factors) should be considered. On the other hand, the report of the Food and Nutrition Board of the National Academy of Sciences, Institute of Medicine *(169)* included Dietary Reference Intakes of 400 μg of dietary folate equivalents, 1.7 mg of vitamin B_6, and 2.4 μg of vitamin B_{12} as an average daily intake. Because a large proportion of the population does not currently meet

these dietary recommendations, a reasonable population approach is to promote an increase in the intake of foods containing these vitamins.

On a population basis, fortification of grains with enough folic acid to deliver 400 µg to the majority of women of childbearing age would be the most logical step to prevent a large fraction of neural tube defects *(170–172)*. The strength of the evidence suggesting that increased folic acid as a public health measure to lower homocysteine might benefit large numbers of elderly people in the prevention of vascular and other disease *(173)* far outweighs the available evidence concerning possible risks for mild neurological impairment with undiagnosed vitamin B_{12} deficiency. Clinical trials have been initiated to assess the role of folic acid for vascular disease prevention with careful attention to its possible neurological effects in vitamin B_{12}-deficient individuals. Meanwhile, action on a population basis should not wait: a combined strategy to increase both folic acid and vitamin B_{12} intake appears prudent at this stage *(156)* and would likely prevent much arteriosclerotic vascular disease as well as neural tube defects.

Despite the fact that fortification seems to have led to higher daily folic acid intakes than anticipated *(166)*, there continues to be wide recognition that the current fortification policy is not optimally lowering homocysteine *(49,174,175)*. There is not consensus regarding the best way to reach this optimum *(153,154)*. We recommend a policy of: (a) fortification of grains with folic acid at a level at least as high as that currently practiced in the United States and (b) the mandatory addition of 1 mg of vitamin B_{12} to all vitamin supplements containing 400 µg of folic acid.

There are population subgroups in which folate requirement may be higher because of a polymorphism in MTHFR or because of regular high alcohol consumption, renal disease, and so forth. For these groups, additional supplementation is likely of even greater importance. While further evidence accumulates, the best course may be to follow physician recommendations tailored to the individual.

On an individual basis, in general, we recommend taking a dietary supplement of 400 µg/d of folic acid to prevent vascular disease. This recommendation has particular potential benefits for middle-aged and elderly individuals, both men and women. Such a dose is found in most multivitamins of the one-a-day type.

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9 n-3 Fatty Acids from Fish and Plants

Primary and Secondary Prevention of Cardiovascular Disease

William E. Connor and Sonja L. Connor

KEY POINTS

- Animal studies, epidemiological studies, and clinical trials have shown that fish and fish oil may reduce sudden death by preventing cardiac arrhythmias.
- n-3 fatty acids have an antithrombotic effect through the diminution of thromboxane A_2 that produces platelet aggregation and vasoconstriction.
- Eicosapentaenoic and docosahexaenoic acids have been shown to inhibit atherosclerosis, probably because of their suppression of cellular growth factors that inhibit the proliferation of smooth muscle cells.
- n-3 fatty acids lower very low-density lipoprotein and triglyceride through depression of synthesis of triglyceride in the liver. n-3 fatty acids also suppress postprandial lipemia, which reduces chylomicron remnants that are considered atherogenic.
- Fish oil does not adversely affect glucose control in patients with diabetes.
- n-3 fatty acids have been uniformly associated with a mild decrease in systolic blood pressure and, at times, a decrease in diastolic blood pressure.
- The intake of n-3 fatty acids should be increased to prevent coronary heart disease.

1. INTRODUCTION

Fish and fish oils contain the very long-chained and highly polyunsaturated n-3 fatty acids (in this chapter, the terms n-3 and omega-3 are used interchangeably), which are derived from phytoplankton, the base of the food chain in oceans, lakes, and rivers *(1)*. Phytoplankton synthesize the n-3 fatty acids eicosapentaenoic (20:5) (EPA) and docosahexaenoic (22:6) (DHA), which are subsequently incorporated into fish, shellfish, and sea mammals. The plants synthesize an n-3 fatty acid, linolenic acid (18:3), which can be converted by the body to EPA and more slowly to DHA *(2,3)*. The n-3 fatty acids have profound biological and biochemical effects in the body. Despite a wealth of scientific information (a review listed more than 120 references about cardiovascular effects alone *(4)*, clinical interest in n-3 fatty acids has not been high in the United States

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despite considerable attention to their use in Europe and Japan. This chapter focuses on the considerable and underappreciated potential benefits of the n-3 fatty acids in cardiovascular disease (CVD).

In the 1950s, it was discovered that polyunsaturated vegetable oils containing the n-6 linoleic acid had a pronounced plasma cholesterol-lowering effect, but the mechanism of this action has remained obscure *(1)*. At that time, it was noted that fish oil, which was also polyunsaturated, had a similar hypocholesterolemic effect. No mention was made of the fact that fish oil contained very long-chain n-3 fatty acids (C20:5 and C22:6) and that these might act differently than the n-6 fatty acids of vegetable oils, such as linoleic acid (C18:2). These early data about fish oil laid fallow until the pioneering observations of Dyerberg and Bang focused special attention on the n-3 fatty acids EPA (20:5) and DHA (22:6), which are found in marine oils *(5)*. Dyerberg and Bang observed a lower coronary mortality among the Greenland Eskimos, whose diet was especially rich in marine oils compared to Danish people, who consumed a diet high in saturated fat *(6)*. It was later discovered that not only did these n-3 fatty acids lower cholesterol, but they had a profound plasma triglyceride-lowering effect, especially in hypertriglyceridemic patients *(7–9)*. Over two decades of research in humans, animals, perfused organs, and tissue cultures have firmly documented the mechanisms of the hypolipidemic actions of these n-3 fatty acids from fish and, furthermore, have demonstrated that these fatty acids have many other beneficial effects in CVD.

This chapter focuses on seven different areas of research that will help to answer the question about the potential benefits of n-3 fatty acids from fish oil for primary and secondary prevention of CVD. These benefits are listed below and are subsequently discussed in detail. We first discuss how n-3 fatty acids will prevent further events in those patients who already have coronary heart disease (CHD) (secondary prevention). This is especially relevant to staving off fatal arrhythmias of the heart and thrombosis. Then, we discuss how n-3 fatty acids might prevent coronary disease in healthy individuals, especially in those who possess certain risk factors.

2. ANTIARRHYTHMIC ACTIONS

2.1. Animal Studies

Sudden death from ventricular arrhythmias is a much-dreaded complication in patients with CHD. Several experimental studies have addressed this problem with the use of n-3 fatty acids from fish oil. McLennan et al. *(10)* used coronary artery ligation in the rat to produce an in vivo model of ventricular fibrillation and myocardial infarction (MI). They found that the number of ventricular ectopic beats and duration of tachycardia or fibrillation was increased in rats that were fed sheep kidney fat (a saturated fat) compared to rats that were fed tuna fish oil, a rich source of n-3 fatty acids. The rats that were fed tuna fish oil had a significantly reduced incidence and severity of arrhythmias. In another animal study, ventricular fibrillation was prevented by fish oil during both the occlusion of the coronary artery and reperfusion of the heart *(11)*.

In other experiments, Hallaq et al. *(12)* used isolated neonatal cardiac myocytes (from hearts of 1-d-old rats) as a model for the study of cardiac arrhythmogenic factors that are modified by n-3 fatty acids. They incubated isolated myocytes (for 3–5 d) in a

culture medium enriched with arachidonic acid or EPA (20:5 n-3). The arachidonic acid-enriched myocytes developed a toxic cytosolic calcium concentration on exposure to ouabain, whereas EPA-enriched myocytes preserved physiological calcium levels. An increase of EPA was demonstrated in the membrane phospholipids, with a small reduction in arachidonic acid in myocytes that were fed EPA. A second study by the same researchers further indicated the mechanism of action of the fish oil fatty acids in preventing the arrhythmias of these isolated myocytes *(13)*. It was found that n-3 fatty acids prevented a calcium-depleted state in the myocytes that was caused by the L-type calcium channel blocker nifedipine. The protective effects of the n-3 fatty acids appeared to result from their modulatory effects on nifedipine-sensitive L-type calcium channels. In a recent study, dogs were given pure EPA, DHA, or α-linolenic acid intravenously *(14)*. Tests were performed in a dog model of sudden death. With infusion of EPA, five of seven dogs did not have a fatal ventricular arrhythmia ($p < 0.02$); with DHA and α-linolenic acid, six of eight dogs in each group were protected ($p < 0.004$ for each). These studies indicated a definite beneficial effect of dietary n-3 fatty acids on the heart; both marine and plant sources of these fatty acids prevented cardiac arrhythmias in animals.

2.2. Population Studies

The epidemiological data regarding dietary n-3 fatty acids and coronary disease are extensive and go back to the initial observations of Dyerberg and Bang, who found a much lower rate of CHD in the Greenland Eskimos compared with Danes *(6)*. By means of extensive studies, they deduced that it was the n-3 fatty acid content of the Eskimo diet that inhibited the atherosclerotic disease despite the fact that the Eskimo diet was a high-cholesterol, high-fat diet *(5)*. Their dietary fat was protective rather than pathogenic, because it was derived from the seas (fish, seal, etc.) and contained the n-3 fatty acids. Furthermore, autopsy studies revealed that the rate of atherosclerosis in Alaskan Eskimos was much less than found in Caucasians living in Alaska *(15)*.

Numerous studies have been conducted correlating fish consumption (providing n-3 fatty acids) and mortality from CHD *(4)*. In Dutch men, the mortality from CHD was more than 50% lower among those who consumed at least 30 g of fish per day than among those who did not eat fish *(16)*. In the Multiple Risk Factor Intervention trial, n-3 fatty acid consumption correlated inversely with all-cause mortality and coronary mortality *(17)*. Even in the Harvard Health Professionals Follow-Up Study of 51,529 men, the consumption of one to two servings of fish per week was associated with a lower incidence of CHD *(18)*. Some of these studies are described in greater detail later.

More recently, fatty fish consumption was associated with prevention of cardiac arrest from ventricular fibrillation in coronary patients. Ventricular fibrillation is the cause of death in most patients with CHD and it accounts for the 20 to 30% of people whose first indication of coronary disease is cardiac arrest. A study from the University of Washington compared the effects of eating fish with the incidence of cardiac arrest *(19)*. There was a 50% reduction in the risk of cardiac arrest in people who consumed at least one fatty fish meal per week. A typical fatty fish would be salmon. Other fatty fish include sardines, mackerel, and Chilean sea bass. Even those who consumed a less fatty fish, such as tuna, benefited, because all fish and shellfish contain the beneficial n-3 fatty acids.

This protection against cardiac arrest occurred from the n-3 fatty acids (EPA and DHA) in the fat of the fish. The effects of eating fish were reflected biochemically in the fatty acids of the red blood cells. Individuals whose red blood cells had a relatively low level of the n-3 fatty acids (3.3% of total fatty acids) had a much greater risk of cardiac arrest than those individuals whose red blood cell n-3 fatty acids comprised 5% or more of the total fatty acids. In other words, there was a 70% reduction in the risk of cardiac arrest in those people with the higher red blood cell n-3 fatty acid content *(19)*. Similarly, the US Physicians Health Study of 20,551 men ages 40 to 84 yr showed a 52% reduction in the risk of sudden cardiac death in men who consumed fish at least once a week *(20)*.

Data from 76,283 women in the Nurses' Health Study showed that a daily intake of 1.1 g or more of α-linolenic acid (18:3 n-3) protected against fatal ischemic heart disease and that this protection probably resulted from an antiarrhythmic effect of α-linolenic acid *(21)*. However, the protective effect of α-linolenic acid did not extend to nonfatal MI, for which there was a nonsignificant trend for an effect.

2.3. Clinical Trials

These epidemiological data are buttressed by a randomized, controlled clinical trial of 2333 men who had recovered from MI and who were then asked to increase their intake of fatty fish or to take fish oil *(22)*. There was a 29% reduction in the 2-yr all-cause mortality in subjects advised to eat fatty fish, and there was also a reduction in deaths from ischemic heart disease, but there was no reduction in nonfatal MI. This was the first intervention trial in which all-cause mortality was reduced in a coronary intervention program. One likely reason for the reduction in coronary mortality was the decrease in cardiac arrest, as documented by Siscovick et al. *(19)*. Men who ate fatty fish at least once a week had a 50% reduction in cardiac arrest, which probably resulted from the antiarrhythmic action of the n-3 fatty acids discussed earlier.

In a second clinical trial, 223 patients with angiographically proven coronary artery disease (CAD) received about 1.5 g/d of n-3 fatty acids from fish oil concentrate for 2 yr *(23)*. The progression of coronary disease was significantly but modestly decreased compared to the control group. Thus, the evidence grew stronger that even some fish (or n-3 fatty acid consumption from fish oil) on a consistent basis (at least one serving a week) may prevent many deaths from CHD.

However, the most recent and largest test of fish oil in coronary patients occurred in Italy; the GISSI-Prevenzione Trial studied 11,324 patients who had survived a recent MI *(24)*. These patients were divided into four groups and were randomly assigned daily supplements of 1 g of n-3 polyunsaturated fatty acids (PUFAs) and 300 mg of vitamin E or no supplements (control). The amount of fish oil used provided approx 0.85 g of EPA and DHA in ethyl esters in a ratio of 1:2. There was no placebo control group. The duration of the supplementation was 3.5 yr. In this massive trial, treatment with the fish oil n-3 PUFAs, but not vitamin E, significantly lowered the risk of death (14–20% less) and of cardiovascular death (17–30% less, dependent on the type of statistical analyses). The deaths and cardiovascular events included nonfatal MI and stroke. Plasma triglycerides were lowered in the patients treated with n-3 PUFAs.

This 1999 study was reanalyzed in 2002 *(25)*. It was shown that the risk of sudden death was significantly prevented by only 3 mo of treatment with fish oil, and there was a 67% reduction in overall deaths. This benefit continued for 3.5 yr to the end of the

study. It is important to recognize that the benefit from fish oil occurred early. Instead of dying from sudden death after the onset of a MI, men treated with fish oil survived in greater numbers.

Several conclusions may be drawn from this study. Fish oil was safely administered over the relatively long time period of 3.5 yr. The authors expected a greater reduction in deaths as occurred in the Diet and Reinfarction Trial in Wales (29%). They attributed their less, but still positive results to a number of coexisting factors. One problem was the relatively low death rate in the control group. It is worth mentioning that all of the patients were consuming the Mediterranean diet, which in itself is associated with a lowered death rate from CHD, perhaps in part from more antioxidant consumption and more fish. A second possible problem was the ratio of EPA to DHA, which in most other studies was 1.5 to 2.0 but in this study was 0.5. EPA may be the more active of the two n-3 PUFAs in its clinical effects (i.e., a direct antagonist of the cyclooxygenase enzyme that produces thromboxane A_2 in platelets). It was believed that the reduction in death rate occurred largely because of the prevention of sudden death from cardiac arrhythmias. This is the first large-scale trial proving that a low dose of fish oil (n-3 PUFAs) over a 3.5-yr time period saved lives. The evidence is steadily mounting regarding the cardioprotective effects of fish oil fatty acids.

The Lyon Diet Heart Study, a randomized secondary prevention trial (following a first MI), showed a remarkable 76% reduction in risk of cardiac death and nonfatal heart attack after 4 yr *(26,27)*. Subjects followed a diet high in canola oil and canola oil margarine (which is high in the plant n-3 fatty acid α-linolenic acid). The diet was also low in fat and high in complex carbohydrates and fiber. Very recent data indicate that α-linolenic acid may have a direct effect on cardiac arrhythmias, whereas other cardiovascular effects are likely mediated through the synthesis of EPA and DHA *(2)*. Conversion of α-linolenic acid to EPA is fairly rapid and measurable within a few days after the dietary ingestion of linolenic acid; the conversion consists of desaturation, elongation, and further desaturation, with the rate-limiting enzyme step being the first desaturation step brought about by Δ^6-desaturase.

The clinical prevention of sudden cardiac death by n-3 PUFAs and the mechanism of this prevention is summarized here *(28)*. Both the clinical and animal studies showing the antiarrhythmic effects of n-3 PUFAs convey exactly the same message, namely, fish oil fatty acids are a powerful but simple modality for prevention of the 300,000 episodes of sudden death that occur annually in the United States. The mechanism of the antiarrhythmic actions is to modulate ion channels, thus stabilizing the cardiac myocytes electrically. Fatty acids act to inhibit the fast, voltage-dependent sodium current and the L-type calcium currents. It was also suggested that the electrical activity in brain neurons is similarly modulated by the n-3 fatty acids quieting action on all excitable tissues, including the neurons of the central nervous system.

3. THROMBOSIS

n-3 fatty acids from fish oil have invariably had an antithrombotic effect, particularly a diminution in thromboxane A_2 that produces platelet aggregation and vasoconstriction *(1,29,30)*. Therefore, platelet reactivity and adhesion were considerably reduced after fish oil ingestion *(31)*. There have been reductions also of plasminogen activator inhibitor-1,

fibrinogen, and tissue plasminogen activator and increases in platelet survival and bleeding time *(4)*. Enhanced fibrinolysis has also been observed *(4)*. Perhaps even more significant was a study in baboons showing that n-3 fatty acids eliminated both vascular thrombus formation and vascular lesions after vascular injury *(32)*. The baboons treated with fish oil showed decreases in thrombus formation at sites of surgical carotid endarterectomy.

The intake of α-linolenic acid either has no effect or leads to decreased platelet aggregation when compared with linoleic acid *(33,34)*. The function of the endothelium, important in both thrombosis and atherosclerosis, is affected by n-3 fatty acids *(29)*. The production of prostacyclin is enhanced, and endothelial-derived relaxation factor, or nitric oxide (which is depressed in atherosclerotic disease), was greatly increased by the n-3 fatty acids of fish oil *(35–37)*. Eight patients with CAD received 1.8 g/d of EPA for 6 wk *(38)*. EPA had beneficial effects on both nitric oxide-dependent and nondependent forearm vasodilitation. The production of prostacyclin increased in rats fed 2.5 and 5% linseed oil *(39)*. Fish oil supplementation improved arterial compliance in diabetic subjects *(40)*. Fish oil supplementation also inhibits norepinephrine-medicated vasoconstriction, which also increases arterial compliance *(41)*. Fifteen obese people with insulin resistance were fed four diets lasting 4 wk each. The diet of 20 g/d of α-linolenic acid (flaxseed oil) *(42)* caused a marked rise in arterial compliance; however, insulin sensitivity and high-density lipoprotein (HDL) cholesterol decreased and low-density lipoprotein (LDL) oxidizability increased.

4. EXPERIMENTAL ATHEROSCLEROSIS AND FISH OIL

When menhaden oil (a fish oil product) was incorporated in atherogenic diets fed to rhesus monkeys, aortic plaques and their cholesterol content were much less than in the groups that were not fed fish oil *(43)*. Because the plasma lipid levels were roughly similar in control groups, the inhibition of atherosclerosis may have involved other mechanisms operative in the vessel wall itself. Carotid atherosclerosis was similarly inhibited. Pigs fed an atherogenic diet had much less coronary atherosclerosis when given cod liver oil containing the n-3 fatty acids *(44)*.

There is good evidence that EPA and DHA from fish oil are even incorporated into advanced human atherosclerotic plaques *(45)*. They are present in complicated plaques as components of cholesterol esters and phospholipids. The incorporation of EPA and DHA from the plasma lipoproteins into the plaques is detectable within a week of fish oil feeding. Perhaps the inhibition of atherosclerosis occurs because EPA and DHA inhibit cellular growth in the arterial wall *(46)*. Atherosclerosis cannot develop even after injury and the influx of LDL cholesterol and cholesterol ester unless there is also a cellular reaction. Two important cells in atherosclerosis are smooth muscle cells and macrophages. Because of the suppression of cellular growth factors by n-3 fatty acids, proliferation of smooth muscle cells was inhibited *(47)*. Likewise, macrophage infiltration into the vessel wall was lessened by n-3 fatty acids *(43)*. Even the initial lesion of atherosclerosis—the fatty streak—develops less under the influence of dietary n-3 fatty acids *(43)*.

5. EFFECTS ON THE PLASMA LIPIDS AND LIPOPROTEINS

There is a major effect on the plasma levels of lipids and lipoproteins as a result of dietary n-3 fatty acids from fish oil *(8,48)*. As shown here, the science in this area is very

clear: n-3 fatty acids in practical doses (<7 g/d) lower the plasma very low-density lipoprotein (VLDL) and triglyceride levels through depression of synthesis of triglyceride in the liver. n-3 fatty acids from fish oil also suppress postprandial lipemia, the chylomicron remnants of which are considered atherogenic. As VLDL concentrations decrease, LDL cholesterol rises, possibly transiently. HDL cholesterol does not change. Similarly to the drug gemfibrozil, n-3 fatty acids may cause an increase in LDL as they lower the plasma triglyceride concentration in some hyperlipidemic states such as familial combined hyperlipidemia; this is discussed in detail later. It is unclear whether n-3 fatty acids from plants (α-linolenic acid) possess these actions. α-linolenic acid from flaxseed oil has not been shown to lower the plasma triglyceride levels, except in very large amounts (38 g/d) *(49)*.

Theoretically, the ideal nutritional program to reduce the plasma lipid and lipoprotein concentrations maximally would be the combination of a diet that is very low cholesterol and saturated fat, which would upregulate the LDL receptor and reduce LDL plasma concentrations, with a diet containing fish oil, which would suppress VLDL production and lower plasma triglyceride concentrations. This view is buttressed by a well-controlled dietary study of fish oil and saturated fat fed as isolated variables. Saturated fat raised the plasma LDL cholesterol levels, and fish oil lowered the plasma triglyceride levels *(50)*.

5.1. Effects of Fish Oil in Normal Subjects

Several recent reviews documented that n-3 fatty acids from fish had a great effect on plasma lipids and lipoproteins, even in normal subjects *(1,8,48)*. The principal action is on the plasma triglyceride and VLDL concentrations. This hypolipidemic action is well-illustrated in a study of 12 healthy adults (6 men and 6 women) who were given three different diets fed in random order for 4 wk each: a saturated control diet, a salmon diet containing considerable amounts of n-3 fatty acids, and a vegetable oil diet high in n-6 fatty acids *(51)*. Both the salmon diet and the vegetable oil diet decreased the plasma cholesterol similarly, from 188 to 162 mg/dL. Both diets reduced LDL from 128 to 108 mg/dL. HDL cholesterol levels were not changed by the salmon diet. The salmon diet decreased VLDL cholesterol levels, and the changes in plasma triglyceride were most striking, from 76 to 50 mg/dL. The polyunsaturated vegetable oils did not lower VLDL and triglyceride levels.

5.2. Studies in Hyperlipidemic Patients

Because of the hypolipidemic effect of n-3 fatty acids from fish oil in normal subjects, it seemed most reasonable to test their effects in hyperlipidemic patients *(9)*. The two groups of hyperlipidemic patients selected for the study were characterized by hypertriglyceridemia, because the depression of the plasma triglyceride and VLDL appeared to be a unique effect of n-3 fatty acids from fish oil.

Twenty hypertriglyceridemic patients volunteered for the study (8 men and 12 women). Of these, 10 presented with increased levels of both VLDL and LDL, consistent with the type II-B phenotype. Their mean plasma lipid levels at time of entry were 337 mg/dL for cholesterol and 355 mg/dL for triglyceride. Clinically, many of these patients had familial combined hyperlipidemia, a disorder characterized by a strong disposition to the development of CHD and by overproduction of lipoproteins, particularly VLDL.

The other 10 patients had apparent type V hyperlipidemia, as characterized by increased chylomicrons and greatly increased VLDL levels in the fasting state. Their mean plasma lipid levels at entry were 514 mg/dL for cholesterol and 2874 mg/dL for triglyceride. Four of the type V patients had concomitant, non-insulin-dependent diabetes mellitus, and two had adult-onset, insulin-dependent diabetes mellitus. Despite the salmon oil, their insulin doses and diabetic control remained constant throughout the study.

Both overproduction of VLDL and impaired clearance of the remnants of chylomicron and VLDL metabolism characterize the type V phenotype. Clinically, type V patients have the "chylomicronemia" syndrome, which is characterized by episodes of abdominal pain from enlargement of abdominal viscera (hepatomegaly and splenomegaly) and episodes of acute pancreatitis. These patients also suffer from eruptive xanthomata, neuropathy, and lipemia retinalis. Although LDL levels are low in patients with fasting chylomicronemia, the presence of the atherogenic remnant particles predisposes them to the development of atherosclerotic complications, including CHD.

Special care was taken to ensure that the patients were in steady-state conditions before entry. Steady-state was defined as a constancy of body weight and diet, and an absence of any residual hypolipidemic drug effect. Most of the patients had not been receiving any hypolipidemic drugs just prior to the study. In the patients previously given drugs, these were discontinued, and plasma lipid levels were monitored until pre-drug levels were attained.

Two different control diets were used for the two groups of hypertriglyceridemic patients, depending on their phenotype of hyperlipidemia. Patients with combined hyperlipidemia (type II-B) received their usual low-cholesterol (100 mg/d), low-fat (20–30% of total calories) diet. Subsequent dietary periods for these patients consisted of a fish oil diet for 4 wk followed, in some patients, by a 4-wk diet that was high in a vegetable oil containing a predominance of n-6 fatty acids. Both of these diets were balanced for cholesterol content (approx 250 mg/d) and contained 30% of calories as fat. The diets in all periods were eucaloric, thus the subjects neither gained nor lost weight.

For patients with fasting chylomicronemia (type V), the control diet consisted of a very low-fat diet (5%) to lower plasma triglyceride levels maximally. The next dietary interval contained fish oil at 20 or 30% of total calories. Finally, a vegetable oil diet that was high in n-6 fatty acids also was provided, which contained 20 to 30% of total calories as fat, and 200 to 300 mg of cholesterol per day. Both the fish oil and the vegetable oil diets initially were used cautiously in the patients with fasting chylomicronemia (type V) to minimize the risk of hepatosplenomegaly, abdominal pain, and acute pancreatitis.

The salmon oil diet provided about 20 g/d of n-3 fatty acids for a 2600-kcal intake, with 30% of total calories as fat. On the other hand, the vegetable oil diet provided about 47 g of the n-6 polyunsaturated fatty acid linoleic acid. Thus, gram for gram, the fish oil diets actually provided 43 to 64% less total PUFAs than the vegetable oil diet.

The fish oil diet decreased the plasma LDL-cholesterol levels in the patients with combined hyperlipidemia (type II-B) by 26 mg/dL. Of individual lipoprotein cholesterol changes, the decline of VLDL cholesterol was most striking; however, LDL and HDL cholesterol also decreased. The plasma triglyceride changes were even greater than the cholesterol changes with the fish oil diet. The plasma triglyceride level decreased from 334 to 118 mg/dL. This occurred largely because of the change in VLDL triglyceride, which was lowered from 216 to 55 mg/dL.

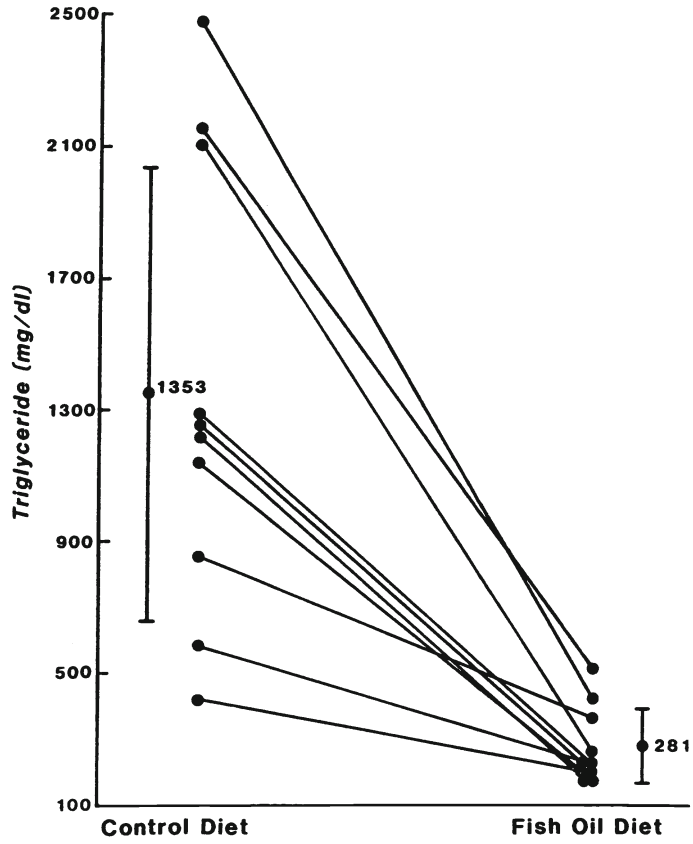

Fig. 1. The changes in plasma triglyceride levels in the 10 type V patients: control diet vs fish oil diet. To convert triglyceride from milligrams per deciliter to millimoles per liter, multiply by 0.0113. Reproduced with permission from ref *9*. ©1985 Massachusetts Medical Society. All rights reserved.

The highly polyunsaturated n-6 vegetable oil diet had a much weaker effect on VLDL cholesterol and triglyceride. LDL values were similar, but, in contrast, HDL cholesterol was higher after the vegetable oil diet. Plasma apolipoprotein changes reflected the lipoprotein lipid changes. In the type II-B patients, there were significant reductions in apolipoprotein (apo)B and C-III levels during the fish oil diet period, which paralleled the declines in LDL and VLDL levels.

The effects of the fish oil diet were even more striking in type V patients (Figs. 1 and 2). With consumption of the very low-fat control diet, their initial plasma lipid levels declined considerably but still remained greatly elevated. Many of these patients still had plasma that appeared milky, with chylomicrons present in the fasting state. The first change to occur in these patients after the fish oil diet was the virtual disappearance of fasting chylomicronemia, which had been present in five of the patients. During the fish oil diet, total plasma triglyceride decreased from a control value of 1353 to 281 mg/dL, a drop of 79% (Fig. 1). VLDL triglyceride decreased similarly, from 1087 to 167 mg/dL. Plasma cholesterol levels declined into the normal range after the fish diet, from 373 to 207 mg/dL (Fig. 2). Most of this total plasma cholesterol decrease occurred as the result of marked changes in the amount of VLDL cholesterol, which decreased from 270 to 70 mg/dL.

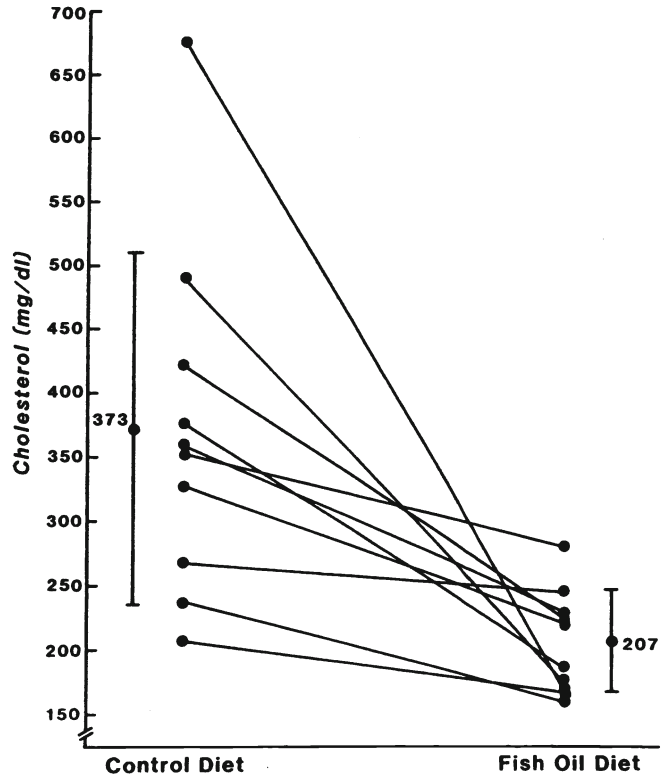

Fig. 2. The changes in plasma cholesterol levels in the 10 type V patients: control diet vs fish oil diet. To convert cholesterol from milligrams per deciliter to millimoles per liter, multiply by 0.026. Reproduced with permission from ref *9*. ©1985 Massachusetts Medical Society. All rights reserved.

Of interest was the 48% concomitant rise of LDL cholesterol, from the low value of 84 to 125 mg/dL. Apo levels changed to reflect the altered lipoprotein lipid levels. Apo A-1 levels did not change, whereas apoB, C-III, and E all decreased significantly.

When the n-6-rich vegetable oil replaced the fish oil in the diets of eight patients, all patients with fasting chylomicronemia (type V) had increases in plasma triglyceride levels within 3–4 d. After 10–14 d of the n-6 vegetable oil feeding, the mean plasma triglyceride values rose 198%, and VLDL triglyceride increased from 171 to 550 mg/dL. Plasma cholesterol also increased, from 195 to 264 mg/dL. However, LDL cholesterol levels were decreased 28% by the vegetable oil diet, again indicating that the metabolic abnormality of the type V phenotype was worsening. Because of enhanced hypertriglyceridemia and the risk of developing abdominal pain typical of this type V disorder, the vegetable oil feeding period was discontinued prematurely in all type V patients *(9)*.

5.3. Implications of the Fish Oil Studies in Hypertriglyceridemic Patients

In the 20 hypertriglyceridemic patients, fish oil incorporated in the diet led to an even more profound hypolipidemic effect than had been observed in normal subjects. The plasma triglyceride levels decreased in each of the 20 patients, with a 79% decrease in the type V patients and a 64% decrease in the patients with combined hyperlipidemia

(type II-B); plasma cholesterol levels decreased 45 and 27%, respectively. In the 12 normal subjects previously investigated *(51)*, decreases were less for plasma triglyceride (38%) and much less for plasma cholesterol (14%). Apparently, the greater the hypertriglyceridemia, the greater the reductions brought about by dietary fish oil—in plasma lipids and especially in VLDL.

These results may have considerable therapeutic importance for patients with severe and moderate hypertriglyceridemia. To date, the only dietary treatment for severely hypertriglyceridemic patients with fasting chylomicronemia (type V) has been the very severe and therapeutically difficult restriction of dietary fat to between 5 and 10% of total calories in an effort to approach normal plasma triglyceride levels. Americans find this possible to do on a short-term basis but very difficult on a long-term basis, because they are accustomed to eating higher quantities of fat (i.e., approx 40% of total calories). Until this study, all fatty foods have been contraindicated in patients with fasting chylomicronemia (type V). The findings of this study suggest that some fatty, and even high-cholesterol, foods (i.e., fish or even shellfish) containing marine n-3 fatty acids are quite appropriate for ingestion and may produce further triglyceride lowering over and above that which results from the very low-fat diet.

Other studies in familial combined hyperlipidemia and in type IV hyperlipidemia showed increases in LDL and apoB while plasma VLDL and triglyceride values declined *(8,52,53)*. Such LDL increases also occurred in type IV patients given the drug gemfibrozil. Perhaps this is an expected physiological action when hypertriglyceridemia is being corrected. Should the LDL levels become abnormally high after administration of drugs or fish oil, then further therapy of the LDL is specifically warranted (i.e., bile acid binding resins or one of the statins, such as lovastatin). Fish oil also produced plasma cholesterol and triglyceride lowering in type III patients and in familial hypercholesterolemia *(54)*.

5.4. Reduction of Postprandial Lipemia After Fatty Meals

It was observed that fish oils markedly decreased the usual chylomicronemia that follows fatty meals *(55,56)*. In other words, fat tolerance was greatly improved (*see* Fig. 3). This improvement could result from diminished absorption, slower synthesis, and slower entry of chylomicrons into the circulation or, alternatively, from a more rapid removal of the chylomicrons that do appear in the circulation. There is no evidence for diminished absorption, and fat balance studies did not show increased fat excretion in stools after dietary periods enriched with fish oil. Whether reduced chylomicron production or enhanced removal of chylomicrons is responsible has not been clarified completely. Fish oil feeding produces smaller VLDL particle size in animals compared to vegetable oil feeding. Therefore, smaller VLDL would have an enhanced catabolism. Perhaps, after a background diet of fish oil, chylomicrons are smaller in size and thus more rapidly catabolized, resulting in a much flatter fat tolerance curve *(55)*.

5.5. The Mechanism of the Hypolipidemic Effects of Fish Oil

The mechanism by which n-3 fatty acids exert their effects to decrease the levels of plasma triglyceride and cholesterol has been tested in humans in two different sets of experiments that studied: (a) the inhibition by fish oil of the usual hypertriglyceridemia that inevitably results when a high-carbohydrate diet is suddenly fed to humans and (b) the effects of fish oil on apoB, VLDL, and LDL production rates and turnovers.

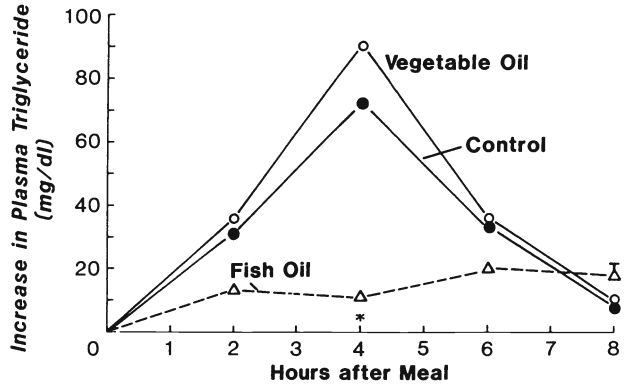

Fig. 3. The increase in plasma triglyceride levels following the ingestion of 50 g of fat. Saturated fat test meal given during the saturated fat diet (closed circles); vegetable oil test meal given during the vegetable oil diet (open circles); salmon oil test meal given during the salmon oil diet (triangles). Mean fasting triglyceride levels were 72 ± 19, 76 ± 37, and 46 ± 11 mg/dL before the saturated fat, vegetable and salmon oil, respectively; test meals were administered.

5.5.1. Fish Oil and the Inhibition of Carbohydrate-Induced Hypertriglyceridemia

The well-known phenomenon of carbohydrate-induced hypertriglyceridemia is a physiological response. In this model, VLDL triglyceride synthesis is stimulated as the dietary carbohydrate intake abruptly increases. The increased VLDL synthesis leads to hypertiglyceridemia, which may persist for many weeks. If n-3 fatty acids do inhibit VLDL synthesis, then the usual carbohydrate-induced hypertiglyceridemia should not occur when fish oil is incorporated into the high-carbohydrate diet.

Seven mildly hypertriglyceridemic but otherwise healthy subjects (ages 22–54 yr) were fed three different experimental diets *(57)*. Each was composed of a liquid formula plus three bran muffins per day to supply fiber. The baseline diet contained 45% of calories from carbohydrates. The high-carbohydrate diets were then divided into control and fish groups; both groups contained 15, 10, and 75% of calories as fat, protein, and carbohydrates, respectively. In the baseline and high-carbohydrate control diets, a blend of peanut oil and cocoa butter provided the fat, which was replaced by fish oil (in the form of a commercially available marine lipid concentrate) in the high-carbohydrate fish oil diet. The total amount of fish oil consumed per day was 50 g (in a 3000-kcal diet), equivalent to approx 3.3 tablespoons of oil. This amount provided 8.5 g of EPA and 5.5 g of DHA.

The three experimental diets were fed in three different sequences in the Clinical Research Center (Fig. 4). In the first sequence, the high-carbohydrate control diet preceded the high-carbohydrate fish oil diet (Fig. 4A). In the second sequence, the high-carbohydrate diet was given for 20 d, rather than 10, to demonstrate that the hypertriglyceridemia did not spontaneously resolve after the first 10 d. This was then followed by the fish oil diet (Fig. 4B). In the third sequence, the fish oil was fed first with the high-carbohydrate diet for 25 d and then removed to permit the effects of the high-carbohydrate diet to be manifest for the next 15 d (Fig. 4C). Three subjects were studied with the first sequence, and two subjects each were studied with the second and third sequences.

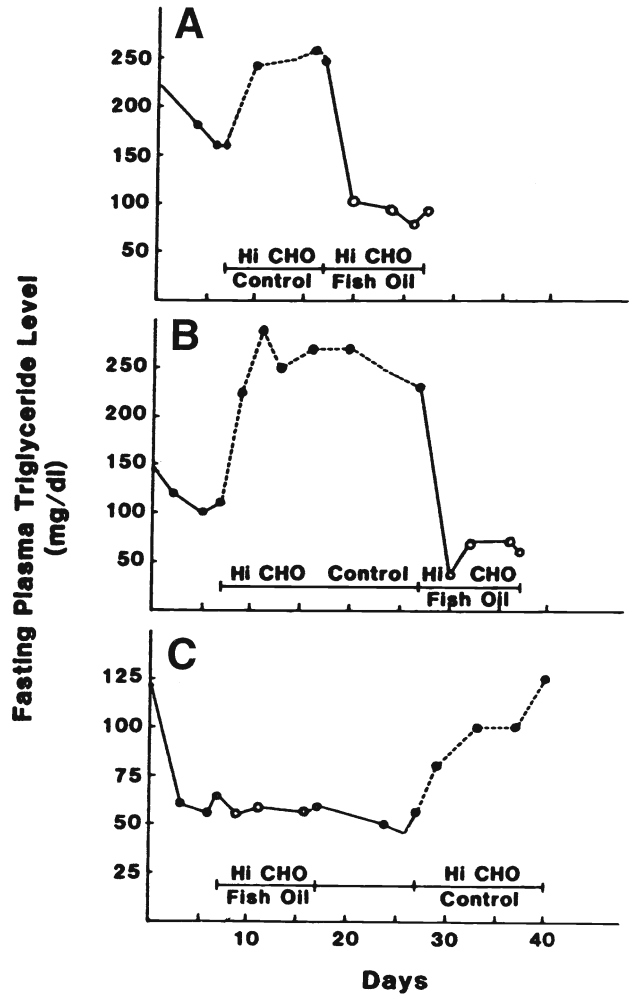

Fig. 4. The effects of the baseline diet, high-carbohydrate (Hi-CHO) control and high-carbohydrate (Hi-CHO) fish oil diets on plasma triglyceride levels in three subjects. We see the reversal of carbohydrate-induced hypertriglyceridemia by dietary fish oil (A), the persistence of the hypertriglyceridemia (throughout 20 d) and the subsequent reversal by fish oil (B), and the prevention of carbohydrate-induced hypertriglyceridemia by fish oil (C).

In all seven subjects, the high-carbohydrate control diet increased the plasma triglyceride levels over the baseline diet, from 105 to 194 mg/dL *(57)*. The magnitude of the carbohydrate-induced hypertriglyceridemia correlated significantly with each individual's baseline triglyceride levels. The rise in plasma triglyceride levels was complete by day 5 and resulted almost entirely from an increase in the VLDL triglyceride fraction, which more than doubled during the control diet, from 69 to 156 mg/dL (Fig. 4). Although the total plasma cholesterol levels did not change, VLDL cholesterol levels almost doubled, from 18 to 34 mg/dL, and HDL cholesterol was reduced, from 49 to 41 mg/dL.

When the fat of the high-carbohydrate control diet was replaced isocalorically with fish oil, the elevated plasma triglyceride concentration was reduced from 194

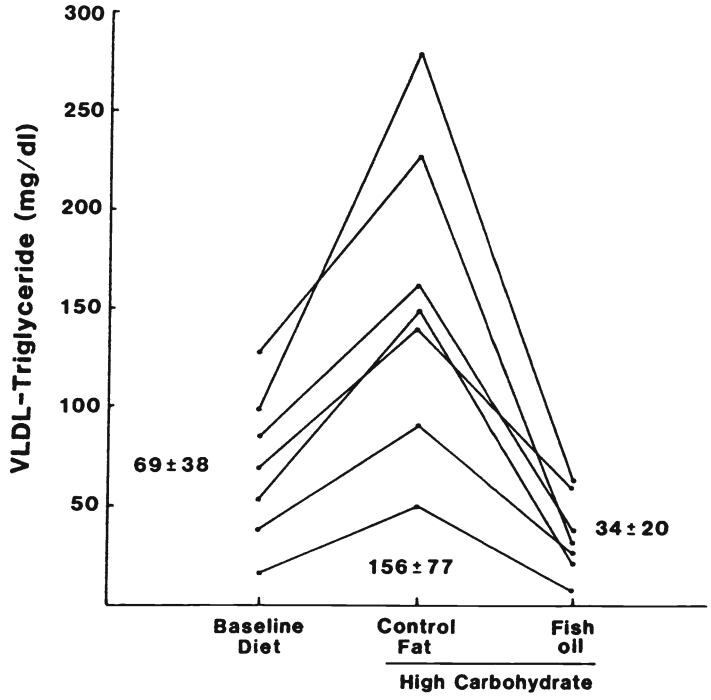

Fig. 5. The effects of the high-carbohydrate control and fish oil diets on the plasma VLDL triglyceride levels in the seven subjects.

to 75 mg/dL, a decrease of 61%. This decrease usually occurred within 3 d (Fig. 4A). Again, changes in VLDL triglyceride levels (from 156 to 34 mg/dL) were largely responsible for this effect (Fig. 5). Total cholesterol levels decreased insignificantly (from 172 to 153 mg/dL) during the high-carbohydrate fish oil diet, primarily because of the drop in VLDL cholesterol levels (from 34 to 12 mg/dL) *(57)*.

The hypertriglyceridemia persisted even when the period of carbohydrate induction was prolonged from 10 to 20 d and did not significantly decrease until fish oil was incorporated into the diet (Fig. 4B). When the high-carbohydrate fish oil diet followed the baseline diet, the plasma triglyceride level did not rise, but the level increased when the high-carbohydrate control diet was fed subsequently (Fig. 4C). The high-carbohydrate control diet decreased the levels of apoB and increased apoC-III concentrations; apoA-1 and E levels did not change. The high-carbohydrate fish oil diet decreased apoA-1 and apoC-III levels; apoB and E concentrations did not change.

The incorporation of corn oil in place of fish oil into the high-carbohydrate regimen failed to prevent the induced hypertriglyceridemia. For the three subjects who participated in this study, the average triglyceride levels were as follows: baseline, 93 ± 23 mg/dL; high-carbohydrate control, 196 ± 58 mg/dL; high-carbohydrate corn oil, 215 ± 90 mg/dL; and high-carbohydrate fish oil, 86 ± 10 mg/dL.

Dietary fish oil not only prevented but also rapidly reversed the dietary, carbohydrate-induced elevations in plasma triglyceride and VLDL levels, whereas the corn oil that was rich in n-6 fatty acids had no effect at all. Because the primary difference between corn oil and the commercial fish oil preparation is the type of polyunsaturated

fatty acids present (corn oil, 57% 18:2 n-6, linoleic acid; the commercially available fish oil preparation, 32% n-3 fatty acids), the difference in effect resulted from the n-3 fatty acids in the fish oil. This finding implied a probable inhibitory effect of n-3 fatty acids on hepatic VLDL production.

5.5.2. Fish Oil and the Synthesis and Turnover of ApoB, VLDL, and LDL

The hypothesis that n-3 fatty acids probably reduced VLDL levels by inhibiting VLDL synthesis was supported by studies designed to elucidate further mechanisms of the hypotriglyceridemic effect of n-3 fatty acids. Dietary fish oil either affected the synthesis or the removal of VLDL. The rates of flux and turnover of VLDL triglyceride were measured after injection of ^{3}H-glycerol into people studied under two dietary protocols: one containing fish oil and the other containing fats typical of the American diet *(58)*. This technique permitted the calculation of both synthetic and removal rates of VLDL.

Ten male subjects were selected because they possessed a wide range of fasting plasma triglyceride concentrations, from 34 to 4180 mg/dL; therefore, the hypothesis about the mechanism of action of dietary fish oils could be tested in subjects with greatly different pool sizes of plasma triglyceride. Liquid formula diets containing 15 to 20% fat, 65 to 75% carbohydrate, and 10 to 15% protein were fed to subjects during both the control and the fish oil dietary periods. The two diets differed only in the type of fat they contained. In the control diet, a blend of cocoa butter and peanut oil (1:2) was incorporated into the formulas. The fish oil diet containing the commercial preparation was taken in three divided doses daily and was not mixed into the formulas. The principal difference between the two diets was the higher content of linoleic acid (18:2 n-6) in the control diet, and the presence of n-3 fatty acids in the fish oil diet. The former diet contained virtually no n-3 fatty acids, whereas the latter provided about 17 g/d of these highly polyunsaturated fatty acids.

The experimental diets were consumed for a period of 3–5 wk before the actual VLDL turnover procedure was conducted. This time was needed for the plasma triglyceride levels to stabilize, particularly in the subjects whose triglyceride levels were above normal. Seven subjects consumed the control diet first, followed by the fish oil diet; in the remaining three subjects, the order was reversed. The order in which the diets were administered did not affect the results.

The isocaloric substitution of fish oil for the control vegetable fat produced the expected significant reductions in the total and lipoprotein lipid levels in all 10 subjects. Total cholesterol levels for all 10 subjects fell from 195 to 144 mg/dL, a reduction of 22%. Decreases in VLDL levels (from 83 to 21 mg/dL) accounted for most of the drop in plasma cholesterol. LDL cholesterol levels did not change significantly, whereas HDL cholesterol concentrations fell from 31 to 24 mg/dL. All of these changes were evident in both the normal and the hypertriglyceridemic groups.

After the administration of ^{3}H-glycerol and its incorporation into the triglyceride of VLDL, the decay curves were analyzed by computer models so that VLDL synthesis and turnover could be calculated. The incorporation of n-3 fatty acids into the diet caused a 72% decrease in the VLDL triglyceride pool size (from 11.4 to 3.2 g; $p < 0.025$). The decreased pool size was associated with a 45% reduction in the VLDL triglyceride synthetic rate (from 23 to 12.6 mg/h/kg ideal body weight [IBW]; $p < 0.005$) and a 45% decrease in the residence time of VLDL triglyceride in the plasma (from 5.8 to 3.2 h; $p < 0.005$). The reciprocal of the residence time is the fractional catabolic rate (FCR),

which was increased by 65% (from 0.23 to 0.38 h-1; $p < 0.005$). There was a significant rise in the cholesterol/triglyceride ratio in VLDL during the fish oil interval (from 0.18 to 0.25; $p < 0.05$). Finally, the ratio of the fast to the slow synthetic pathways did not change with fish oil feeding. The same trends were seen in both normal and hypertriglyceridemic patients. Similar results were also found by using a slightly different dietary plan and with the labeling of VLDL apoB with ^{125}I *(59)*. There was a striking reduction of VLDL synthesis and enhanced turnover.

Direct evidence that the hepatic synthesis of triglyceride and VLDL is suppressed by n-3 fatty acids from fish oil was produced in three in vitro studies of the perfused rat liver and in studies of liver cells from rats and rabbits in primary culture *(60–62)*. In all of these studies, triglyceride synthesis was reduced. In one, enhanced ketone body production resulted; in the others, there was a diversion of n-3 fatty acids from triglyceride synthesis into phospholipid synthesis *(62)*. When the net results of the human and animal studies are taken together, the evidence is very strong that suppression of VLDL and triglyceride synthesis is a primary mechanism for the hypolipidemic effects of n-3 fatty acids, coupled with an increased FCR of VLDL.

5.6. Fish Oil and LDL Turnover

Labeled LDL turnover studies have been carried out in normal subjects given fish oil. One of these studies demonstrated that there was a decreased synthesis of LDL and a tendency for an increased FCR *(63)*. Spady and colleagues *(64)* showed enhancement of LDL receptor activity after the administration of fish oil in the rat. This latter result is consistent with the increased FCR observed in the normal human subjects. It seems clear that the n-3 fatty acids from fish oil have major effects on all of the major lipoproteins, with the exception of HDL.

5.7. Summary and Conclusions: Fish Oil Effects on Plasma Lipids and Lipoproteins

The n-3 fatty acids from fish oil and fish have been shown to have a remarkable effect on the synthesis and clearance of triglyceride-rich lipoproteins, especially VLDL and chylomicrons. Even LDL synthesis and clearance have been affected. Because of these significant effects on lipoprotein synthesis and clearance, beneficial effects of fish oil have been demonstrated in a variety of hyperlipidemic states, especially those with conditions such as hypertriglyceridemia and chylomicronemia. Therapeutic implications for fish oil are especially positive in hyperlipidemia types V, IV, and III. A similar effect has been shown in hypertriglyceridemic diabetic patients without affecting glucose homeostasis *(65)*.

There has been difficulty in interpreting the effects of fish oil in various hyperlipidemic patients because of vastly different experimental conditions *(8)*. In some studies, fish oil was simply added as a supplement to the usual diet in doses of 8 to 16 g/d. In the control period, a placebo oil (such as olive oil or safflower oil) was not always used. In other studies, there was the customary diet plus the use of an appropriate placebo oil. Furthermore, various kinds of fish oils have been used, some containing a considerable amount of cholesterol and saturated fat. Newer fish oils have less saturated fatty acids, higher concentrations of n-3 fatty acids, and a lower cholesterol content.

Some conclusions have emerged from the wide variety of studies, most of which have not been controlled for caloric and body weight stablility. Fish oil is most effective when the diet is well-controlled. In these studies, LDL lowering usually has occurred as well

as profound VLDL and triglyceride lowering in normal subjects and in a wide variety of hyperlipidemic states. In our experience, this lowering of plasma cholesterol levels has occurred in patients with elevated plasma triglyceride concentrations (types V, II-A, II-B, III, and IV hyperlipidemia), with the most dramatic results occurring in the patients with fasting chylomicronemia (type V) who do not tolerate any other kind of dietary fat *(8,9,48,66)*. In the literature and in our experience, HDL levels have not been greatly affected by fish oil. Clearly, the use of fish oil in hyperlipidemia must be individualized regarding both use and dosage. Lower doses of fish oil (8–15 g/d) or 2.4–4.5 g of n-3 fatty acids (EPA and DHA) particularly lower the plasma triglyceride levels *(8)*.

Why has there been an occasional increase in the plasma LDL and apoB after fish oil administration when there has been a simultaneous decrease in plasma VLDL and triglyceride? This is a most challenging question and may relate to fundamental aspects of VLDL-LDL metabolism. Normally, LDL is derived from two sources: conversion from VLDL and direct synthesis from the liver. Similarly, the catabolism of VLDL occurs in two directions through intermediate density lipoprotein. This lipoprotein may be removed by the apoE receptor in the liver or converted to LDL. The animal experiments of Huff and Telford *(67)* suggest the reason that in some instances, fish oil might increase LDL. Turnover studies in the miniature pig revealed that fish oil feeding increased the proportion of VLDL being converted to LDL. Apparently, the n-3 fatty acids of fish oil produce a smaller VLDL particle, which is more likely to be converted to LDL. In this pig study, however, LDL concentrations did not increase, because the direct synthesis of LDL was reduced more by fish oil than the increase in LDL from VLDL. The findings from these pig studies await confirmation in humans. They do explain the reason that LDL may increase in some humans who are fed fish oil: more VLDL is converted to LDL and direct LDL synthesis does not decrease, thus there is more LDL. LDL turnover studies showed decreased production of LDL in normal humans given large amounts of salmon oil vs vegetable oil *(63)*. In this study, the plasma LDL decreased after n-3 fatty acids.

6. FISH OIL IN PATIENTS WITH DIABETES

In patients with diabetes, there is enhanced risk for vascular disease, so that the use of fish oil might be particularly desirable if glucose control is not disturbed. The literature is controversial regarding the effects of n-3 fatty acids in patients with diabetes *(68,69)*. In type I, insulin-dependent diabetics, there is universal agreement that glucose control is not hampered with fish oil supplementation and that the beneficial effects on the plasma lipids and lipoproteins have been demonstrated. In type II, adult-onset non-insulin-dependent diabetics, the results have been somewhat conflicting, possibly because such patients are very susceptible to the caloric load imposed. In most studies, the plasma triglyceride and VLDL concentrations have declined, but some studies have shown the deterioration in glucose homeostatsis. This literature was reviewed recently by Heine and colleagues *(68)*. Most of the studies were short-term and, in some, caloric control has been somewhat distorted by the administration of calorie-dense fish oil without the existence of a suitable placebo. The addition of fish oil to the usual diet would be hypercaloric, thereby disturbing glucose control. When there is attention to the caloric content of the supplement, then there are beneficial effects on the plasma triglyceride and VLDL without disturbing glucose homeostasis, as illustrated in the following experiments.

These problems and objections were considered in an experimental design of a study of 16 adult-onset diabetics who were randomized to a double-blind, placebo-controlled, crossover study *(65)*. The subjects of the study were overweight, and most were receiving hypoglycemic agents. There was a 3-mo stabilization baseline period in which they were given a eucaloric, lower fat (30% of calories from fat), high-complex carbohydrate diet with 55% of calories from carbohydrate. This was followed by two 6-mo intervention periods in which the subjects continued on the same diet and received a supplement of 15 g/d of either olive or fish oil. The fish oil contained 6 g/d of n-3 fatty acids. The endpoints of the study were plasma lipid and lipoprotein concentrations and glucose homeostasis. The plasma triglyceride concentrations were much lower with the fish oil preparation vs olive oil (260 vs 449 mg/dL). VLDL cholesterol and VLDL triglyceride were lower. The total plasma cholesterol was unchanged. There was a significant increase in LDL cholesterol, from 117 to 145 mg/dL, when hypertriglyceridemic individuals were given fish oil. HDL cholesterol did not change.

However, the effects of fish oil on glucose homeostasis revealed no difference from the 6-mo period of olive oil administration. Body weights were unchanged. Fasting glucose levels were 172 and 178 mg/dL for olive and fish oil, respectively. Another measure of diabetic control, hemoglobin A-1 C, revealed no difference nor did the 24-h urinary glucose excretion, the plasma C-peptide, or the 24-h urinary C-peptide.

In view of the extremely high mortality from CHD in adult-onset diabetics, this hypolipidemic action of fish oil was of interest because diabetic control did not deteriorate, and there were significantly beneficial plasma lipid lipoprotein effects. The other actions of the n-3 fatty acids from fish oil in inhibiting the development of atherosclerosis, preventing thromboxane A_2 formation, increasing endothelial-derived relaxing factor, and inhibiting platelet-derived growth factor are all additional reasons for postulating a therapeutic benefit from the use of fish oil in patients with diabetes.

7. HYPERTENSION

Eleven studies showed a uniform association between n-3 fatty acids and a mild decrease in systolic blood pressure and, at times, a decrease in diastolic blood pressure, particularly in the upright position *(4)*. This has especially occurred in mild hypertensives *(4,70,71)*. A randomized controlled trial was conducted to study the effects of fish intake and weight loss on blood pressure in medication-treated, overweight hypertensive patients *(72)*. Combining a daily fish meal with weight loss resulted in additive decreases in ambulatory blood pressure and decreased heart rate. The suggested mechanism has been an attenuation in the responses of forearm vascular resistance and bloodflow to angiotensin (i.e., less vascular reactivity). Because the decreases in both systolic and diastolic pressures are not large even in the best studies (4.6 mmHg and 3.0 mmHg, respectively) *(71)*, fish oil cannot be regarded as a single treatment modality for hypertension. However, when used for other purposes, the mild blood pressure-lowering effect of n-3 fatty acids would certainly provide an added benefit.

8. CONCLUSION

The intake of n-3 fatty acids from fish and plants should definitely be increased to prevent CHD *(2,73)*. This could be best implemented in the form of two to three fish

meals per week in the context of a low-fat diet. Fish, of course, could be substituted for meat in the diet. An intake of 2 g/d of α-linolenic acid or 1% of energy has been suggested *(73)*. At the same time, the diet should be reduced in fat content to 20% of the total calories with a high intake of carbohydrates and fiber. The intake of cholesterol should be limited to 100 mg/d.

Table 1 provides the fat and n-3 fatty acid content for a wide variety of fish and shellfish *(74)*. All fish and shellfish contain the n-3 fatty acids even when the fat content is rather low, as seen in shellfish. The lower the fat content, the higher the percentage of n-3 fatty acids that are present in a given fish or shellfish. The goal of this recommendation is to produce an increased content of the n-3 fatty acids EPA and DHA in the blood and tissues of the body. This will occur if there is regular consumption of fish and shellfish (at least to the extent of 200–300 g/wk) such that these n-3 fatty acids will be present in the diet.

With the depletion of wild fish stocks, consumers will more commonly find only farmed fish on the markets, especially salmon and catfish. An important issue involves the EPA and DHA content of farmed fish. Preliminary data from our laboratory indicate that EPA and DHA are 50 to 75% lower in farmed catfish. Farmed and wild salmon have similar amounts of EPA and DHA. Additional analyses are needed to confirm these data.

Table 2 provides the n-3 fatty acid (α-linolenic acid) content from various oils and foods *(2)*. Daily consumption of 15 g (1 Tbsp.) of canola oil or canola oil margarine provides as much as 1 g of α-linolenic acid per day. Other rich dietary sources of α-linolenic acid include English walnuts and flaxseed oil. α-linolenic acid also is a prominent fatty acid of green leafy vegetables; however, because these vegetables have such a low fat content, the net amount of α-linolenic acid ingested from these sources is small. Human, but not cow, milk is a good source of the n-3 fatty acids, including α-linolenic acid, EPA, and DHA. The amounts in human milk (2.2% of total fatty acids) provide a good basis for considering similar amounts in the diets of children and adults. In recognition of the importance of n-3 fatty acids in human nutrition, the producers of infant formulas are including soy oil, an excellent source of α-linolenic acid, as one of the fat ingredients in formula, rather than corn and coconut oils, which are poor sources. Similarly, intravenous fat preparations use soy oil instead of safflower oil, which is a poor source of n-3 fatty acids.

If researchers desire to ascertain the long-term intake of fish and shellfish, there are excellent markers to document this point. The markers would be: the measurement of the n-3 fatty acids in the plasma, which would reflect a more immediate intake; their measurement in red blood cells, which, because of the greater half-life of these cells, would reflect the intake over a longer period of time; and finally, biopsies of the adipose tissue in which fatty acids would reflect the intake over many months and years *(75)*.

For the intensive treatment of various forms of hyperlipidemia as well as the production of an antithrombotic state, fish oils would need to be used in addition to the consumption of fish. The dose of fish oil might well be from 6 to 15 g/d, titrated according to the endpoint desired.

For people who are unable to consume fish or shellfish, the use of fish oil would again be advisable. For primary prevention, 2 to 3 g/d would be desirable. As noted earlier, higher doses should be used for secondary prevention and the attainment of discrete endpoints of plasma lipid and lipoprotein levels and platelet function.

Table 1
Fat and n-3 Fatty Acid (EPA and DHA) Content of Seafood and Fish Oils

Seafood (100 g, edible portion, raw)	*Fat (grams)*	*n-3 fatty acids (EPA & DHA) (grams)*
Fish		
Anchovy, European	4.8	1.4
Bass, striped	2.3	0.8
Bluefish	6.5	1.2
Carp	5.6	0.3
Catfish, channel	4.3	0.3
Cod, Atlantic	0.7	0.3
Cod, Pacific	0.6	0.2
Flounder, unspecified	1.0	0.2
Haddock	0.7	0.2
Halibut, Pacific	2.3	0.4
Herring, Atlantic	9.0	1.6
Herring, Pacific	13.9	1.7
Mackerel, Atlantic	13.9	2.5
Mullet, unspecified	4.4	1.1
Perch, ocean	1.6	0.2
Pike, walleye	1.2	0.3
Pompano, Florida	9.5	0.6
Sablefish	15.3	1.4
Salmon, Atlantic	6.3	1.2
Salmon, Chinook	10.4	1.4
Salmon, pink	3.4	1.0
Salmon, sockeye	8.6	1.2
Sardines, in sardine oil[a]	15.5	3.3
Snapper, red	1.2	0.2
Sole	1.2	0.1
Sturgeon	3.3	0.3
Swordfish	2.1	0.2
Trout, brook	2.7	0.4
Trout, lake	9.7	1.6
Trout, rainbow	3.4	0.5
Tuna	2.5	0.5
Crustaceans		
Crab, Alaska King	0.8	0.3
Crab, Dungeness	1.0	0.3
Crayfish, unspecified	1.4	0.1
Lobster, Northern	0.9	0.2
Shrimp, unspecified	1.2	0.3
Mollusks		
Abalone, New Zealand	1.0	Trace
Clam, hardshell	0.6	Trace
Clam, littleneck	0.8	Trace
Mussel, blue	2.2	0.5
Octopus, common	1.0	0.2
Oyster, Pacific	2.3	0.6
Scallop, unspecified	0.8	0.2
Squid, unspecified	1.1	0.3

[a]Analysis by the Atherosclerosis Research Laboratory, Portland, OR.

Table 2
α-Linolenic Acid Content of Various Oils and Foods

Food	*α-Linolenic acid % by wt*
Oils	
Flaxseed oil	50.8
Canola oil	9.3
Soy oil	7.0
Corn oil	1.0
Olive oil	0.6
Safflower oil	0.4
Chocolate	0.1
Coconut oil	Trace
Nuts	
English walnuts, dry roasted	6.8
Almonds, dry roasted	0.4
Hazelnuts, dry roasted	0.2
Cashews, dry roasted	0.2
Peanuts, dry roasted	Trace
Green, leafy vegetables, raw (edible portion)	
Brussels sprouts	0.20
Kale	0.13
Spinach	0.12

9. RECOMMENDATIONS

The n-3 fatty acids from fish, fish oil, and plants greatly inhibit the atherosclerotic process, coronary thrombosis, and cardiac arrhythmias by a variety of actions and should be considered as an important therapeutic modality in patients with already established CHD and to prevent coronary disease in highly susceptible people.

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10 Antioxidant Vitamin Supplementation and Cardiovascular Disease

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KEY POINTS

- Free radical-related processes underlie many of the proinflammatory and vascular cell changes associated with atherogenesis.
- Although observational studies have been mixed, the most consistent inverse association between antioxidant vitamin intake and reduced cardiovascular disease (CVD) has been reported with vitamin E.
- Primary and secondary randomized controlled trials, including arterial imaging trials, have predominantly indicated a null effect of antioxidant vitamin supplementation on CVD events and progression of atherosclerosis.
- Troublesome data from randomized controlled trials (RCTs) of antioxidant vitamin supplementation have arisen in certain subgroups.
- Limited data indicate that low antioxidant levels may be a prerequisite for the cardioprotective effects of antioxidant vitamin supplementation to be expressed.
- Current data do not support the use of antioxidant vitamin supplementation for the prevention or treatment of CVD.
- The divergent information derived from observational trials and RCTs warrants further investigation.
- Consumption of fruits and vegetables high in antioxidant vitamins appears to be an important component of a healthy lifestyle.

1. INTRODUCTION

A large body of laboratory evidence suggests that the early stages of atherosclerosis are comprised of a series of oxidative processes *(1)*. Animal studies suggest that oxidative damage may be involved with atherogenic-promoting processes, such as endothelial damage *(2)*, and that antioxidants reduce oxidative damage and atherosclerosis lesions *(3,4)*. Therefore, there is much basic science research and animal studies supporting the antiatherogenic hypothesis of antioxidants. Although information concerning antioxidants

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and human atherosclerosis is accumulating, the relevance of this hypothesis to atherosclerosis prevention in humans remains unproven.

Studies in humans indicate an association between blood measures of oxidative processes and atherosclerosis *(5)*. These data are paralleled by observational studies, including arterial imaging studies *(6,7)* that have demonstrated an inverse association between use of antioxidant vitamin supplements and cardiovascular disease (CVD). Data from randomized controlled trials (RCTs) have been less consistent, and the safety of certain antioxidant supplementations has been questioned in some studies. This chapter reviews evidence from observational studies, RCTs, and atherosclerosis arterial imaging studies to determine whether there is an anti-atherogenic effect of antioxidant vitamins in humans.

2. FREE RADICALS, OXIDATION, ANTIOXIDANTS, AND ATHEROSCLEROSIS

Oxidative processes that evoke early atherogenesis resemble mechanisms described for lipid and protein oxidation by free-radical species. Relative to free-radical-mediated injury to cells and tissues, certain antioxidants provide significant scavenging and protective effects. It has been suggested that oxidative damage to tissues is the outcome of an imbalance between free-radical species formation and elimination. Accordingly, emphasis has been placed on antioxidant interventions. Different categories of antioxidant defenses have been described, and are subdivided into nonenzymatic and enzymatic antioxidants *(8)*. A detailed overview of free-radical mechanisms that are relevant to the modification of lipoproteins and injury to vascular tissues has been published *(9)*.

Lipid peroxidation, a free-radical-related process, underlies many of the proinflammatory and vascular cell changes associated with atherogenesis. Mechanisms to prevent lipid peroxidation have been the focus of antioxidant research. Free-radical-related mechanisms initiating lipid peroxidation in tissues and lipoproteins involve a host of reactive oxygen species (ROS), including superoxide anion radicals (O_2^-) and H_2O_2, both formed metabolically and by redox cycling of many compounds *(10)*. Metabolic sources for ROS include enzymes of the mitochondrial respiratory chain *(11)*, xanthine oxidase *(12)*, the cytochrome P450 enzyme family *(13)*, the nicotinamide adenine dinucleotide/ nicotinamide adenine dinucleotide phosphate oxidases *(12)* and lipoxygenases *(14)*. The reactivity of ROS and rate of lipid peroxidation is facilitated by certain transition metals that are probably complexed to proteins, because free transition metals do not exist in normal tissues at catalytic levels sufficient to support the ROS production rates needed to induce cellular lipid peroxidation. Iron or copper bound to protein serve as effective catalysts, especially with saturation of protein-binding sites *(15)*. This has been described for ceruloplasmin, which has been implicated in the oxidation of serum lipoproteins *(16)*. Plausible mechanisms for initiating lipid peroxidation may also involve heme proteins, which can be converted to potent oxidants *(17)*.

Interaction of ROS with nitric oxide (NO) represents another important pathway for oxidation reactions between cells and lipoproteins. Oxidation reactions resulting from the interaction of O_2^- with NO not only produce ROS (notably peroxynitrite) but consume NO that normally functions to prevent peroxidation of lipids and proteins *(18)*. Thus, antioxidant properties of NO may be lost along with other important vascular functions. Notably, vitamin E treatment improves endothelium-dependent vasomotor capacity and prevents decreased expression of endothelial NO synthase (NOS) in hypercholesterolemic

animals *(19)*. This protective effect is thought to be based on the ability of vitamin E to reduce peroxynitrite formation and spare NO *(20)*.

Certain steps of arterial wall cellular metabolism that lead to atheroma formation can be modulated by low-density lipoprotein (LDL)-derived oxidation products. The type and proportion of oxidation products are dependent on the radical-generating system. Cu^{2+}-mediated oxidation is a common in vitro system used to investigate the role of LDL components during lipid peroxidation. It is also used in vitro to determine LDL oxidative susceptibility in healthy subjects and in patients in states associated with oxidative stress, such as myocardial infarction (MI). Measurements often are based on the inhibition period of oxidation (lag time), typically using 5 to 10 μmol of Cu^{2+}/LDL-cholesterol (LDL-C). The susceptibility of LDL to oxidation is dependent on the composition of LDL (i.e., on its antioxidant, lipid hydroperoxide, and fatty acid content) *(21)*. Cu^{2+}-oxidized LDL (Ox-LDL) shares similar properties with LDL isolated from arterial lesions *(21)*.

Oxidation of lipoproteins, particularly LDL, forms a heterogeneous group of unsaturated carbonyls that react with the lysine ε-amino groups of apolipoprotein B (apoB), thus reducing the proportion of positively charged amino acids *(22)*. LDL accordingly acquires a progressively negative net charge and is taken up by a receptor that binds negatively charged apoB, scavenger receptor-A1 *(23)*. A continuum of increasingly Ox-LDL can be formed based on the duration and/or severity of oxidation. A threshold for oxidative modification of LDL apoprotein has been proposed on the basis of the number of covalently modified lysine groups *(24)* and by modifications at other domains of the apoprotein *(25)*. Progressive modifications along important domains of apoB, particularly at the ligand epitopes for cell LDL receptors, reduce its capacity for assimilation by receptor-mediated endocytosis *(25)*. Ox-LDL can exist as both soluble and insoluble (aggregate) particles when LDL is oxidized with Cu^{2+} to varying degrees *(26)*.

Lipid hydroperoxides formed in lipoproteins react with apoproteins via two mechanisms: (a) by decomposition to reactive carbonyls, or (b) by addition of the peroxy radical to free amino groups, including those found in phosphatidylethanolamine *(27)*. A reaction of the hydroperoxide with protein has been proposed to involve release of O_2^-, which may participate in further ROS-mediated oxidation reactions. This second mechanism may account for more fluorescent products after LDL oxidation than are found by reactions with aldehydic products. This reaction may take place in parallel with formation of Schiff bases, which are common reaction products of reactive carbonyls. The spectrum for the fluorescent products for reactions, via either mechanism 1 or 2, resembles the spectrum obtained for Ox-LDL that is isolated from atherosclerosis tissues or LDL subjected to a variety of oxidation reactions. This suggests that similar reactions may occur during the oxidation of lipoproteins in vivo.

Oxidized lipoproteins are known to be cytotoxic to vascular cells *(28)*, with toxicity attributable to the lipid components *(29)*. The cytotoxic effects appear to result from a free-radical-mediated oxidation process induced in cells upon exposure to Ox-LDL, because toxicity is prevented mostly by antioxidant pretreatments *(30)*. Receptor-mediated delivery of the oxidized lipoproteins provides a targeted means for delivering oxidized lipids and their decomposition products to intracellular sites, resulting in the signaling and expression of stress-response genes, cytokines, and adhesion molecules. Ox-LDL is chemotactic to monocytes and inhibits macrophage motility, effects also attributable to the lipid components *(31,32)*. Further evidence that these effects result from oxidized lipids comes from studies *(33)* showing that lipid loading and binding to collagen are

inhibited by antioxidants. Studies using the antioxidants probucol and butylated hydroxytoluene offer compelling, but indirect, evidence for LDL oxidation as an important factor contributing to atherogenesis *(3,4)*. In atherosclerosis animal studies *(3,4)*, these agents slowed the development of atherosclerosis . These atherogenic events may be evoked either directly or indirectly through the presence of lipid peroxidation products and, in a concerted manner, facilitate the development of an atherosclerosis lesion. The ability to induce the expression of cytokine genes, along with a series of other acute response proteins in vascular cells, is shared by both Ox-LDL and fatty acid hydroperoxides. Treatment of endothelial cells with either oxidant results in augmented expression of cytokine-mediated formation of adhesion molecules (vascular cell adhesion molecule and intercellular adhesion molecule) *(30)*. Inhibition of these responses by antioxidants is taken as evidence that the effects occur through cellular oxidant stress involving redox-sensitive transcriptional or posttranscriptional factors *(34)*. Similarly, the induction of factors such as monocyte chemotactic peptide is evoked by Ox-LDL and lipid peroxidation products through oxidant-sensitive transcriptional factors *(34)*. In this respect, vitamin E—specifically R,R,R-α-tocopherol—may have a modulatory role on signal transduction events associated with the regulation of smooth muscle cell proliferation. The effect appears to be unrelated to its direct antioxidant activity (i.e., inhibition of lipid peroxidation and free-radical generation), although an indirect action related to modulating the cellular redox state is plausible. It has been proposed *(35)* that protein kinase C (PKC)-α inhibition by α-tocopherol is linked to the activation of a protein phosphatase, which in turn dephosphorylates PKC-α and inhibits its activity. This mechanism of action may not only influence the signaling events regulating smooth muscle cell proliferation but may also inhibit formation of early atherosclerosis lesions, demonstrated in rabbits in which vitamin E prevented cholesterol-induced atherosclerosis lesions and the induction of PKC-α activity *(36)*. Therefore, vitamin E appears to operate at several levels, either by direct inhibition of oxidative modification of lipoproteins or cell lipids or by indirect effects that may be independent of its antioxidant activity. Moreover, there may be interaction between these two mechanisms that would likely obscure responses that are monitored in vivo. An example can be taken from the well-known inhibition of LDL oxidation and the consequent reduction of this ligand for the CD36 receptor *(37)*. Decreased ligand binding would, in turn, affect signaling events such as activation of nuclear factor-κB *(38)* and peroxisome proliferator-activated receptor *(39)* transcription factors, which modulate a variety of cell responses. In each of these cases, the oxidant-sensitive/antioxidant-inhibitable mechanism involves activation of transcription factors that signal cell proliferation, chemotaxis, and adhesion of inflammatory cells at vascular loci where atherosclerosis lesions are prone to develop. The extent to which specific antioxidants are able to modulate these responses is currently under investigation.

3. OBSERVATIONAL EVIDENCE FOR ANTI-ATHEROGENIC EFFECTS OF ANTIOXIDANTS

3.1. Ecological Studies

Several early ecological studies that reported an inverse correlation between consumption of vitamin-containing foods and CVD suggest a possible role of dietary antioxidants in the prevention of atherosclerosis . In Scotland and England, fresh fruit

and vegetable consumption was inversely associated with CVD mortality *(40,41)*. Vitamin C from fresh fruit and vegetable consumption was inversely related with coronary heart disease (CHD) mortality *(40,41)*. Data from the US Department of Agriculture and vital statistics from 1964 to 1978 indicated an inverse correlation between fruit and vegetable consumption and CVD mortality, which was especially true for foods rich in vitamin C *(42)*. A similar correlation was observed between increased ascorbate production and reduction in CHD mortality in the United States between 1958 and 1978 *(43)*. Although these represent important observations, results from these ecological studies are limited, because they are susceptible to large interpretative biases resulting from the inability to link CVD risk with food consumption in specific individuals. Furthermore, these studies were not adjusted for concomitant CVD risk factors.

3.2. Epidemiological Studies

3.2.1. Vitamin E Dietary and Supplementary Intake

Five large-scale prospective cohort studies *(44–48)* investigated the relationship between vitamin E intake and CVD (Table 1). Although only one of these studies *(46)* found decreased CHD risk associated with dietary intake of vitamin E, three studies found that supplementary vitamin E intake was significantly associated with lower CHD risk in the elderly *(48)*, middle-aged women *(44)*, and men *(45)*. The Nurses' Health Study was the largest of these studies, with 87,245 female nurses (ages 34–59 yr) initially free of CHD who completed dietary questionnaires that assessed daily dietary and supplementary antioxidant vitamin intake *(44)*. During a follow-up of 8 yr, 552 cases of CHD were documented, including 437 nonfatal MIs and 115 coronary deaths. Compared to women in the lowest quintile of vitamin E intake (<3.5 IU/d), those in the top quintile (>21.5 IU/d) had a relative risk (RR) of CHD of 0.66 (95% confidence interval [CI], 0.50–0.87; $p < 0.001$), after adjustment for age, smoking, and a variety of additional CVD risk factors. The CHD benefit was attributable primarily to supplementary vitamin E intake, because dietary sources were not associated with significant reductions in CHD. Those who used supplementary vitamin E for less than 2 yr had no significant reduction in CHD risk (RR = 0.86, 95% CI, 0.52–1.43). However, use of supplementary vitamin E for 2 yr or more was associated with a significant reduction in CHD risk, after adjustment for CVD risk factors and other antioxidant vitamins (RR = 0.59, 95% CI, 0.38–0.91). Women taking at least 100 IU/d of specific vitamin E supplements (i.e., not multivitamins) had a relative risk of CHD of 0.57 (95% CI, 0.41–0.78) compared with nonusers. There was no trend toward a greater decrease in CHD risk with increasing daily intake of supplementary vitamin E; relative risk was 0.56 (95% CI, 0.21–1.51) for an intake of 100–250 IU/d, 0.56 (95% CI, 0.33–0.96) for intake of 300–500 IU/d, and 0.58 (95% CI, 0.24–1.42) for intake greater than 600 IU/d.

In the Health Professionals Follow-Up Study, 39,910 male health professionals (ages 40–75 yr) who were initially free of CVD completed dietary questionnaires that assessed daily dietary and supplementary antioxidant vitamin intake *(45)*. During a 4-yr follow-up, 667 cases of CHD were documented, including 360 coronary artery bypass grafts or percutaneous transluminal coronary angioplasties, 201 nonfatal MIs, and 106 fatal MIs. Compared with men in the lowest quintile of vitamin E intake (median intake: 6.4 IU/d), those in the top quintile (median intake: 419 IU/d) had a relative risk of 0.64 (95% CI, 0.49–0.83; $p < 0.003$) for CHD after adjustment for age, smoking, body mass index, hypertension, and additional CVD risk factors and a relative risk of CHD of 0.60

Table 1
Epidemiological Studies of Dietary and Supplementary Vitamin Intake

Vitamin E
Nurses' Health Study
Health Professionals Follow-Up Study
Iowa Women's Health Study
Finnish Mobile Clinic Study
Established Populations for Epidemiologic Studies of the Elderly
Vitamin C
Nurses' Health Study
Health Professionals Follow-Up Study
Iowa Women's Health Study
Finnish Mobile Clinic Study
Established Populations for Epidemiologic Studies of the Elderly
Alameda County Study
Western Electric Study
Swedish Women's Study
British Department of Health and Social Security Nutritional Survey
First National Health and Nutrition Examination Survey Epidemiologic Follow-Up Study
β-carotene
Nurses' Health Study
Health Professionals Follow-Up Study
Iowa Women's Health Study
Finnish Mobile Clinic Study
Western Electric Study
Massachusetts Health Care Panel Study

(95% CI, 0.44–0.81; $p < 0.01$) with further adjustments for vitamin C and carotene intake. The CHD benefit was primarily attributable to supplementary vitamin E intake, because dietary sources were not associated with significant reductions in CHD. For men consuming 60 IU or more of vitamin E per day, the RR of CHD was 0.64 (95% CI, 0.49–0.83) compared to those consuming less than 7.5 IU/d. Maximal reduction in risk was seen with an intake of 100–249 IU of vitamin E per day, with no further decrease in risk at higher intakes. Men taking at least 100 IU of vitamin E supplements per day for 2 yr or longer had a relative risk of 0.63 (95% CI, 0.47–0.84) for CHD compared with nonusers, after controlling for multivitamin use. Men taking at least 100 IU of specific vitamin E supplements (i.e., not multivitamins) per day had a relative risk of 0.75 (95% CI, 0.61–0.93) for CHD compared with nonusers.

The Iowa Women's Health Study followed 34,486 postmenopausal women for up to 7 yr *(46)*. The women (ages 55–69 yr) were initially free of CVD and completed questionnaires that assessed daily dietary and supplementary antioxidant vitamin intake. During follow-up, 242 women died of CHD. In the overall cohort, vitamin E intake from food and supplements was not associated with a lower risk of CHD death (RR = 0.96, 95% CI, 0.62–1.51; $p = 0.27$). However, vitamin E intake from food was inversely associated with CHD death among the subgroup of 21,809 women who did not consume vitamin supplements. Compared with women in the lowest quintile of vitamin E intake (<4.91 IU/d), those in the top quintile (>9.64 IU/d) had a relative risk of 0.38 (95% CI, 0.18–0.80; $p < 0.004$) for CHD death after adjustment for age, total energy intake, and

a variety of additional CVD risk factors. Unlike the Nurses' Health Study *(44)* and the Health Professionals Follow-Up Study *(45)*, supplementary intake of vitamin E was not associated with lower CHD risk. Intake of foods rich in vitamin E (such as margarine, nuts and seeds, and mayonnaise or creamy salad dressings) was inversely associated with a lower risk for CHD death.

The Finnish Mobile Clinic Study was conducted in 5133 Finnish men and women (ages 30–69 yr) who were initially free of CVD *(47)*. Daily dietary and supplementary antioxidant vitamin intake was assessed with questionnaires at baseline. During the mean 14-yr follow-up period, there were 186 CHD deaths in men and 58 CHD deaths in women. In the 2748 men, those in the highest tertile of vitamin E intake had a relative risk of 0.68 (95% CI, 0.42–1.11; p-trend = 0.01) for CHD death relative to those in the lowest tertile after adjustment for CVD risk factors. In the 2385 women, those in the highest vs lowest tertile of vitamin E intake had a relative risk of 0.35 (95% CI, 0.14–0.88; p-trend < 0.01) for CHD death after adjustment for CVD risk factors. Individuals who died of CHD consumed more dairy products and less vegetables, fruits, and margarine than did the survivors. The adjusted relative risk for CHD mortality was 0.55 (95% CI, 0.18–1.73) among the 3% of men and women who used supplements containing vitamin E or vitamin C vs those individuals who did not use supplements.

The Established Populations for Epidemiologic Studies of the Elderly was a prospective study designed to determine the effect of vitamin E and C supplement intake (not part of a multivitamin) on CHD mortality in 11,178 men and women ages 67 to 105 yr *(48)*. During the 6 to 9 yr of follow-up, there were 3490 deaths, including 1101 CHD deaths. Vitamin E supplement intake was associated with a relative risk of 0.59 (95% CI, 0.37–0.93) for CHD mortality as well as a relative risk of 0.73 (95% CI, 0.58–0.91) for all-cause mortality after adjustment for CVD risk factors. However, the dosages of the vitamin supplements and consistency of their use were not assessed.

3.2.2. Vitamin C Dietary and Supplementary Intake

Ten large-scale prospective cohort studies *(45–54)* examined the relationship between vitamin C intake and CVD (Table 1). The results from these studies have been less consistent than those for vitamin E, and the majority of the studies do not support a relationship between vitamin C intake and CVD protection. Eight of these studies *(45–52)* found no CVD risk reduction from vitamin C intake. After adjustment for other vitamin and multivitamin intake, both the Nurses' Health Study *(49)* and the Health Professionals Follow-Up Study *(45)* showed no additional CVD benefit from vitamin C intake. In the Health Professionals Follow-Up Study, men in the top quintile of vitamin C intake (median intake: 1162 mg/d) vs the lowest quintile (median intake: 92 mg/d) had a nonsignificant decreased risk for CHD (RR = 0.89, 95% CI, 0.68–1.16) *(45)*. In the Iowa Women's Health Study, women in the top quintile of vitamin C intake (>196.3 mg/d) vs the lowest quintile (<87.3 mg/d) had a nonsignificant increased risk for CHD mortality (RR = 1.43, 95% CI, 0.75–2.70) *(46)*.

In the Finnish Mobile Clinic Study, women in the highest vs lowest tertile of vitamin C intake had a relative risk of 0.49 (95% CI, 0.24–0.98; $p = 0.06$) for CHD death after adjustment for CVD risk factors; men had a relative risk of 1.00 (95% CI, 0.68–1.45; $p = 0.94$) *(47)*. In the Established Populations for Epidemiologic Studies of the Elderly, vitamin C supplement vs no supplement intake was associated with a relative risk of CHD mortality of 0.99 (95% CI, 0.74–1.33) after adjustment for CVD risk factors *(48)*.

In the Alameda County Study, 3119 men and women (ages 16 yr and older) were followed for up to 10 yr *(50)*. The subjects completed dietary questionnaires that assessed daily dietary and supplementary antioxidant vitamin intake. During follow-up, 276 deaths occurred. There were no significant relationships between vitamin C intake above 250 mg/d and mortality from cancer, CVD, or total mortality.

In the Western Electric Study, 1556 men (ages 40–55 yr) were followed for 24 yr *(51)*. Although there was a relative risk of 0.75 (95% CI, 0.52–1.07) for CHD death in the highest tertile of vitamin C intake (>112 mg/d) vs the lowest tertile (<83 mg/d), this was not statistically significant. In a 12-yr follow-up study of 1462 Swedish women (ages 38–60 yr), vitamin C intake, estimated from 24-h dietary recalls, was not associated with CVD mortality, MI, or stroke after controlling for CV risk factors *(52)*.

In the British Department of Health and Social Security Nutritional Survey, 730 British men and women (ages 65 yr and older) were followed for up to 20 yr *(53)*. The subjects were initially free of CVD and completed 7-d dietary records. During follow-up, there were 124 deaths from stroke and 182 deaths from CHD. Compared with subjects in the lowest tertile of vitamin C intake (≤ 27.9 mg/d), those in the top tertile (≥44.9 mg/d) had a relative risk of 0.50 (95% CI, 0.30–0.80; $p < 0.003$) for death from stroke after adjustment for age, gender, and CVD risk factors. In contrast to the stroke results, there was no significant association between vitamin C intake and CHD mortality (RR = 0.8, 95% CI, 0.6–1.2).

The First National Health and Nutrition Examination Survey Epidemiologic Follow-Up Study was the only large-scale prospective cohort study to find a significant CVD benefit from vitamin C intake *(54)*. This study involved a median follow-up of 10 yr in 11,348 men and women (ages 15–74 yr). In the cohort of 4479 men, there were 1069 verified deaths, and in the 6869 women, there were 740 verified deaths. Men who had a dietary intake of vitamin C of no less than 50 mg/d and who consumed vitamin C supplements on a regular basis had a CVD standardized mortality ratio of 0.58 (95% CI, 0.41–0.78) compared with all US Caucasian males, for whom the standardized mortality ratio was defined to be 1.00. Women with the same vitamin C intake had a CVD standardized mortality ratio of 0.66 (95% CI, 0.53–0.82). The average vitamin C supplement intake among users was 800 mg/d. The inverse association between vitamin C intake and CVD mortality appeared to be explained by supplementary intake, because vitamin C dietary intake was not significantly associated with reduced CVD mortality. However, caution in the interpretation of these results is warranted, because the use of other vitamin supplements or multivitamins was not considered in the data analyses.

3.2.3. β-Carotene Dietary and Supplementary Intake

Six large-scale prospective cohort studies *(45–47,51,55,56)* investigated the relationship between β-carotene intake and CVD (Table 1). As in the case with vitamin C, the reported relationships between β-carotene intake and CVD were inconsistent. Of the six reported prospective cohort studies, only two showed a statistically significant inverse relationship between β-carotene intake and CVD *(45,55)*. Although women in the highest vs lowest quintile of β-carotene intake had a RR of 0.78 (95% CI, 0.59–1.03) for CHD risk in the Nurses' Health Study *(56)*, this did not reach statistical significance after adjustment for age, smoking, and other CHD risk factors. In the Iowa Women's Health Study, no association was found between CHD mortality and vitamin A, retinol, or carotenoids from dietary or supplement intake alone or from dietary and

supplement intake combined *(46)*. In the Finnish Mobile Clinic Study, women in the highest vs lowest tertile of β-carotene intake had a relative risk of 0.62 (95% CI, 0.30–1.29; $p = 0.60$) for CHD death after adjustment for CVD risk factors; men had a relative risk of 1.02 (95% CI, 0.70–1.48; $p = 0.36$) *(47)*. Although there was a relative risk of 0.79 (95% CI, 0.60–1.04) for CHD death in middle-aged men in the highest (>15.9 mg/d) vs the lowest (<2.9 mg/d) tertile of β-carotene intake in the Western Electric Study, this did not reach statistical significance *(51)*.

In the Health Professionals Follow-Up Study, men in the top quintile of carotene intake (dietary and supplementary median intake: 19,034 IU/d) vs the lowest quintile (median intake: 3969 IU/d) had a lower risk for CHD (RR = 0.71, 95% CI, 0.53–0.86; $p = 0.03$) *(45)*. Although this inverse relationship between carotene intake and CHD risk was not found among those men who had never smoked (RR = 1.09, 95% CI, 0.66–1.79; $p = 0.64$), an inverse association was apparent among current smokers (RR = 0.30, 95% CI, 0.11–0.82; $p = 0.02$) and former smokers (RR = 0.60, 95% CI, 0.38–0.94; $p = 0.04$).

The Massachusetts Health Care Panel Study examined the association between consumption of carotene-containing fruits and vegetables and CVD mortality in a prospective cohort of 1299 Massachusetts residents (ages 66 yr and older) who were initially free of CVD *(55)*. During a 4.75-yr follow-up, 161 cases of CVD mortality were documented, including 48 confirmed fatal MIs. Compared with men and women in the lowest quartile of carotene-containing fruit and vegetable consumption (<0.8 servings/d), those in the top quartile (≥2.05 servings/d) had a relative risk of 0.59 (95% CI, 0.37–0.94; $p = 0.014$) for CVD death after adjustment for age, smoking, cholesterol intake, alcohol consumption, and additional CVD risk factors and a relative risk of 0.27 (95% CI, 0.10–0.74; $p = 0.005$) for fatal MI.

3.3. Studies Measuring Serum Antioxidant Concentrations

In general, studies examining the relationship between serum antioxidant concentrations and CVD have been inconclusive *(57–74)*. Specifically, studies examining the relationship between serum β-carotene, vitamin A, and vitamin C concentrations and CVD have been as inconsistent as the observational studies examining CVD and dietary and supplementary intake of these antioxidant vitamins. Also, studies examining the relationship between serum vitamin E concentrations and CVD have not consistently confirmed the observational studies examining dietary and supplementary intake of vitamin E and CVD.

3.3.1. Cross-Sectional Studies

In the cross-sectional World Health Organization/Multinational Monitoring Project of Trends and Determinants of Cardiovascular Disease Study (WHO/MONICA), multiple groups of approx 100 healthy men (ages 40–49 yr) from 16 different worldwide regions differing sixfold in age-standardized CHD mortality were compared regarding plasma levels of vitamins A, C, E, and carotene *(57,58)*. Independent of lipid levels, the plasma vitamin E concentration was the strongest inverse predictive factor for cross-cultural CHD mortality rates. This study also indicated that the low vitamin E plasma levels may have a greater impact on CHD mortality than classical risk factors, such as hypercholesterolemia or hypertension. Across all 16 regions, where LDL-C levels ranged from 3.36 mmol/L (130 mg/dL) to 4.91 mmol/L (190 mg/dL), moderate associations between CHD mortality rates and plasma cholesterol ($r^2 = 0.29$; $p = 0.03$) and diastolic blood pressure

($r^2 = 0.25$; $p = 0.05$) were found; a considerably stronger inverse association was found for lipid-standardized vitamin E plasma levels ($r^2 = 0.62$; $p = 0.0003$). Lipid-standardized vitamin A ($r^2 = 0.24$; $p = 0.05$), vitamin C ($r^2 = 0.11$; $p = 0.22$), and carotene ($r^2 = 0.04$; $p = 0.48$) plasma levels showed weaker inverse associations with CHD mortality rates. Among 12 regions with common plasma LDL-C levels, ranging from 3.88 mmol/L (150 mg/dL) to 4.40 mmol/L (170 mg/dL), the absolute levels of vitamin E ($r^2 = 0.63$; $p = 0.002$) and lipid-standardized vitamin E ($r^2 = 0.73$; $p = 0.0004$) showed strong inverse correlations with CHD mortality rates. Additionally, vitamin C plasma levels showed a moderately strong, statistically significant inverse correlation with CHD mortality rates among these 12 regions ($r^2 = 0.41$; $p = 0.03$). Lipid-standardized vitamin A ($r^2 = 0.16$; $p = 0.19$) and carotene ($r^2 = 0.21$; $p = 0.14$) plasma levels showed no association with CHD mortality rates among the 12 regions. In regions with low and medium CHD risk (Italy, Switzerland, and Northern Ireland), the average vitamin E plasma levels among healthy middle-aged males were 26 to 28 μmol/L; in regions with the highest CHD risk (Finland and Scotland), vitamin E plasma levels were 20 to 21.5 μmol/L *(58)*. On average, vitamin E plasma levels were about 25% lower ($p < 0.01$) in regions with high CHD risk than in regions of low-to-medium risk, and there was only a small overlap in the distributions of vitamin E plasma levels. This indicates that there may be a threshold of CHD risk from vitamin E plasma levels below 25 to 30 μmol/L *(58)*. A similar threshold for CHD risk may be operational for vitamin C plasma levels below 23 to 51 μmol/L *(58)*. In a smaller cross-sectional survey *(59)* of four European regions, the evidence did not support the hypothesis that plasma antioxidant concentrations explain regional differences in CHD mortality.

In a cross-sectional survey *(60)* of 595 individuals (ages 50–84 yr) conducted in an urban population of India, a significant inverse association was found between plasma vitamins A, C, E, and β-carotene levels and prevalence of coronary artery disease (CAD; defined as presence of angina or diagnosis of MI). The vitamin A and E levels remained inversely related to CAD after adjustment for other CVD risk factors. The adjusted odds ratio (OR) for CAD, between the lowest and highest quintiles of plasma vitamin levels in 523 subjects without CAD and 72 subjects with CAD, was significant only for plasma vitamin E levels (OR = 2.53, 95% CI, 1.11–5.31); the lowest quintile plasma vitamin E levels were below 11.8 μmol/L, and the highest quintile plasma vitamin E levels were greater than 19.2 μmol/L.

The relation between serum ascorbic acid level and the prevalence of CVD was analyzed among 6624 US men and women (ages 40–74 yr) enrolled in the National Health and Nutrition Examination Survey *(61)*. Compared with participants with the lowest serum vitamin C concentration (≤ 0.4 mg/dL), there was a 27% (95% CI, 10–41%) decreased prevalence of CHD and a 26% (95% CI, 3–44%) decreased prevalence of stroke after adjustment for CVD risk factors obtained from demographical and historical information among those participants with the highest (≥1.1 mg/dL) serum vitamin C concentration. Other CVD risk factors, such as plasma cholesterol levels and other serum antioxidant levels, were not controlled for in the analyses. In addition, misclassification of CVD may have occurred, because this diagnosis was self-reported.

3.3.2. Case–Control Studies

Case–control and prospective cohort studies have yielded mixed results and, in general, have not confirmed the inverse relationship between serum vitamin antioxidant levels and

CVD found in cross-sectional/ecological studies. Three nested case–control studies, conducted within large prospective cohort studies, were reported *(62–64)*. In two of these studies, no association was found between serum vitamin E or vitamin A concentrations and subsequent CVD mortality *(62,63)*. However, in the nested case–control studies from the Netherlands *(62)* and eastern Finland *(63)*, blood samples were collected at baseline, and vitamin assays were determined 7 to 10 yr after sampling. Blood samples were frozen at −20°C, a temperature at which antioxidant vitamins are unstable and undergo degradation. Thus, it is probable that the measured vitamin E levels were inaccurate. In the nested case–control study from Washington County, Maryland, blood samples were properly frozen at −70°C and analyzed for vitamin levels 16 yr after collection *(64)*. Although no association was found between serum R,R,R-α-tocopherol levels and risk for MI in the total sample, a protective association for MI was suggested with higher serum levels of α-tocopherol in the subgroup of individuals with serum cholesterol levels of 6.21 mmol/L or higher (≥240 mg/dL). In addition, an increasing risk for MI was found with decreasing serum β-carotene concentrations ($p = 0.02$), with a similar trend found with decreasing levels of lutein ($p = 0.09$). However, the excess risk for MI associated with low serum carotenoid concentrations was limited to current smokers.

In a case–control study of 110 cases of angina pectoris and 394 controls selected from a sample of 6000 men (ages 35–54 yr), a significant inverse association was found between vitamin E plasma levels and angina pectoris *(65)*. Vitamin E levels remained independently and inversely associated with the risk of angina pectoris after adjustment for age, smoking, blood pressure, lipids, and weight. The adjusted odds ratio for angina pectoris between the lowest vs highest quintiles of vitamin E plasma levels was 2.68 (95% CI, 1.07–6.70; $p = 0.02$). Adjusted plasma levels of vitamin C, vitamin A, and carotene were inversely, but not significantly, associated with angina pectoris. Several double-blind RCTs examining the effect of vitamin E administration on angina pectoris yielded mixed results *(75–77)*.

3.3.3. Prospective Cohort Studies

At least three prospective cohort studies *(66–68)* reported the relationship between serum vitamin E, vitamin C, and β-carotene concentrations and CVD risk. The Basel Prospective Study examined the relationship between baseline serum vitamin C, vitamin E, and β-carotene concentrations and subsequent CVD mortality in 2974 male Swiss pharmaceutical company employees (ages 41–59 yr) who did not initially have CVD *(66)*. During 12 yr of follow-up, 553 men died, including 132 from ischemic heart disease (IHD) and 31 from stroke. Compared with men in the highest quartile of serum carotene (β-carotene plus α-carotene) concentration, those in the lowest quartile (<0.23 μmol/L) had a relative risk of 1.53 (95% CI, 1.07–2.20; $p < 0.024$) for CVD mortality after adjustment for age, smoking, blood pressure, and cholesterol. Compared with men in the highest quartile of serum vitamin C concentration, those in the lowest quartile (<22.7 μmol/L) had a relative risk of 1.25 (95% CI, 0.77–2.01; $p = 0.38$) for CVD mortality. Separately, low serum concentrations of carotene and vitamin C were not associated with death from stroke, with relative risks of 2.07 (95% CI, 0.78–5.46; $p = 0.14$) and 1.28 (95% CI, 0.40–4.09; $p = 0.34$), respectively. However, the risk of death from stroke was significantly increased in subjects who were in both the lowest quartile of carotene (<0.23 μmol/L) and vitamin C (<22.7 μmol/L) plasma levels, relative to those in the highest quartile of both carotene and vitamin C (RR = 4.17, 95% CI, 1.68–10.33; $p = 0.002$). There was no association between CVD mortality and serum vitamin E concentrations. This latter finding may result from the

fact that the median serum vitamin E concentration in the cohort was 35 μmol/L, and, as such, all of the serum vitamin E concentration quartiles were above the presumed threshold for CHD risk of 25–30 μmol/L, as suggested by the WHO/MONICA cross-sectional study *(58)*. This also may have been true for the vitamin A levels.

The relationship between serum carotenoid concentration and subsequent CHD events (nonfatal MI and CHD death) was prospectively assessed in 1899 hyperlipidemic men assigned to the placebo arm of the Lipid Research Clinics Coronary Primary Prevention Trial *(67)*. A total of 1883 men (ages 40–59 yr) had data for serum carotenoid levels and smoking status. After a 13-yr follow-up, men in the highest quartile of serum β-carotene concentration (>3.16 μmol/L) had a relative risk of 0.64 (95% CI, 0.44–0.92; $p = 0.01$) for CHD events compared with those in the lowest quartile (<2.33 μmol/L) after adjustment for age, smoking, high-density cholesterol, low-density cholesterol, and other known CHD risk factors. This finding was stronger among the 441 men who never smoked, in whom the relative risk was 0.28 (95% CI, 0.11–0.73; p-trend = 0.06). For the 679 current smokers, the relative risk was 0.78 (95% CI, 0.44–1.34; p-trend = 0.04).

The relationship between serum vitamin C concentration and subsequent MI was prospectively assessed in 1605 randomly selected men in eastern Finland with an average age of 54 yr who were initially free of CVD *(68)*. After up to 8.75 yr of follow-up, there were 70 fatal or nonfatal MIs. Compared with men in the highest quintile of plasma vitamin C concentration (>64.8 μmol/L), men in the lowest quintile (<11.4 μmol/L) had a relative risk of 4.03 (95% CI, 1.74–9.36; $p = 0.001$) for fatal or nonfatal MI after adjustment for age, season, and examination year. However, after adjustment for a variety of CVD risk factors, the RR was substantially diminished to 2.08 (95% CI, 0.82–5.30) and was no longer statistically significant.

Taken together, the studies relating serum antioxidant vitamin concentrations with CVD have been inconsistent and inconclusive. These studies further complicate the inconsistencies seen with the vitamin C and carotene dietary intake studies and, in general, have failed to confirm the apparent protective effect of high dietary and supplementary intake of vitamin E.

3.4. Arterial Imaging Studies

3.4.1. Angiographical Studies

In contrast to the relatively large number of epidemiological studies examining the association between antioxidant vitamin intake and CVD, antioxidant relationships with atherosclerosis have been studied infrequently through direct arterial visualization. Leukocyte ascorbic acid levels were significantly lower in patients with angiographically proven CAD than in patients without CAD *(69)*. Vitamin E plasma levels were equal between patients with and without angiographically proven CAD, but plasma levels were no less than 30 μmol/L in both groups *(70)*. In another study, plasma total R,R,R-α-tocopherol levels were not different between patients with and without angiographically proven CAD; LDL R,R,R-α-tocopherol levels were unexpectedly significantly higher in patients with angiographically proven CAD *(71)*. These studies are difficult to interpret because no details of angiographic results were provided, and the classification systems used for categorizing patients with and without angiographically proven CAD were too imprecise to determine associations with risk factors. Additionally, the plasma antioxidant levels measured after diagnosis may have reflected lifestyle changes.

The first data relating antioxidant vitamin intake with progression of atherosclerosis came from the Cholesterol Lowering Atherosclerosis Study (CLAS) *(6,7)*. CLAS was a randomized, placebo-controlled, serial arterial imaging clinical trial designed to determine whether drug reduction of LDL-C would reduce the progression of atherosclerosis in the coronary *(78,79)*, carotid *(80,81)*, and femoral *(82)* arteries, as determined from serial angiographic and ultrasonographic measurements of change in atherosclerosis *(83)*. Ancillary analyses of dietary and supplementary antioxidant vitamin intake used a large database derived from 7-d food records collected on the 188 CLAS subjects every 2 mo *(83)*. The association between self-selected dietary and supplementary antioxidant vitamin intake and progression of CAD was derived from analysis of the serial quantitative coronary angiographic data from CLAS *(6)*. Overall, subjects with supplementary vitamin E intake of no less than 100 IU/d demonstrated less coronary artery lesion progression (average per-subject change in percent diameter stenosis [%S]) than subjects with supplementary vitamin E intake of less than 100 IU/d for all lesions (–0.8 vs 2.0 %S; $p < 0.04$) and for mild/moderate lesions less than 50%S (–0.6 vs 3.1%S; $p < 0.02$). Among the subjects randomized to colestipol-niacin treatment in whom LDL-C was reduced to less than 2.59 mmol/L (100 mg/dL), a benefit of supplementary vitamin E intake was found for all lesions (–1.1 vs 3.4%S; $p < 0.03$) and mild/moderate lesions (2.2 vs 3.2%S; $p < 0.02$). Although placebo subjects with supplementary vitamin E intake of 100 IU/d or greater demonstrated less coronary artery lesion progression than placebo subjects with supplementary vitamin E intake of less than 100 IU/d for all lesions (1.7 vs 2.9%S) and mild/moderate lesions (1.8 vs 4.0%S), these differences were not significant. Dietary vitamin E intake was inversely, but nonsignificantly, associated with progression of CAD. No benefit was found for intake of dietary or supplementary intake of vitamin C. Because CAD progression (specifically, progression of lesions less than 50%S) is linked to the risk of clinical coronary events *(84)*, CLAS results present a plausible explanation for reduced CHD events seen with supplementary vitamin E intake in other studies.

Angiographic studies examining the effects of supplementary antioxidant vitamins for preventing restenosis after cardiac angioplasty have been less successful. In a double-blind, placebo-controlled trial, 115 subjects (average age 54 yr) were randomized to either 400 IU of α-tocopherol three times a day or placebo 48 h after angioplasty *(85)*. Four months after therapy, 36% of subjects who received R,R,R-α-tocopherol had restenosis vs 48% of subjects who had received placebo ($p = 0.06$).

In the Multivitamins and Probucol (MVP) Trial *(86)*, 317 subjects (mean age: 60 yr) were randomly assigned to one of four treatments: probucol (500 mg), multivitamins (700 IU of vitamin E, 500 mg of vitamin C, and 30,000 IU of β-carotene), both probucol and multivitamins, or placebo. The agents were administered to subjects twice daily 4 wk before and 6 mo after angioplasty; subjects were also given an extra 1000 mg of probucol, 2000 IU of vitamin E, both probucol and vitamin E, or placebo 12 h prior to the angioplasty, according to their treatment assignments. Six months after angioplasty, the mean ± standard deviation (SD) reduction in luminal diameter was 0.12 ± 0.41 mm in the probucol-treated group, 0.22 ± 0.46 mm in the combined probucol-multivitamin group, 0.33 ± 0.51 mm in the multivitamin group, and 0.38 ± 0.50 mm in the placebo-treated group. For those subjects receiving probucol vs those not receiving probucol, these results were significant ($p = 0.006$); for those subjects receiving vitamins vs those

not receiving vitamins, these results were not significant ($p = 0.70$). Restenosis occurred in 25.0, 35.9, 42.2, and 42.9% of the subjects treated with probucol, the combination of probucol and multivitamins, just multivitamins, and placebo, respectively. For those subjects receiving probucol vs those not receiving probucol, these results were significant ($p = 0.004$); for those subjects receiving vitamins vs those not receiving vitamins, these results were not significant ($p = 0.49$). It is not clear why probucol (a potent antioxidant) prevented restenosis, whereas multivitamin antioxidants—either alone or in combination with probucol—did not. Results similar to those of the MVP Trial were reported from the Probucol Angioplasty Restenosis Trial, another trial of probucol vs placebo *(87)*.

3.4.2. B-Mode Ultrasound Studies

High-resolution, B-mode ultrasound measurement of arterial wall intima-media thickness (IMT) determines the extent and progression of subclinical atherosclerosis in its earliest stages and can be used to determine atherosclerosis relationships in asymptomatic individuals *(88–91)*. Furthermore, because antioxidants are hypothesized to affect atherosclerosis at its earliest stages, carotid wall measurements are ideal for determining associations with the earliest clinical stages of atherosclerosis. Several reports have investigated the association between carotid artery IMT and antioxidant vitamin intake and their plasma levels.

In the largest cross-sectional arterial imaging study *(92)*, the average carotid artery IMT was determined by quintile of R,R,R-α-tocopherol, ascorbic acid, and carotene dietary intake in 6318 women and 4989 men (ages 45–65 yr) without clinical evidence of atherosclerosis. Vitamin intake was adjusted for age, race, body mass index, LDL-C, HDL-C, smoking, plasma glucose, blood pressure, and caloric intake. Among women and men younger than age 55 yr, there was no association between dietary intake of any of the antioxidant vitamins and carotid wall thickness. Among women age 55 yr and older, those consuming more dietary vitamin C or α-tocopherol had less carotid wall thickening (p-trend across quintiles of vitamin intake were 0.019 and 0.033, respectively). Among men age 55 yr and older, those consuming more dietary vitamin C had less carotid wall thickening (p-trend across quintiles was 0.035). No significant associations were found between antioxidant vitamin supplement intake and carotid wall thickness. Serum vitamin antioxidant levels from 231 cases (defined as subjects exceeding the 90th percentile for carotid IMT) and 231 controls (defined as subjects below the 75th percentile for carotid IMT) were examined from the same cohort *(72)*. Cases with a thicker carotid artery IMT had significantly lower levels of the specific carotenoids β-cryptoxanthin and lutein plus zeaxanthin. However, after adjustment for other CVD risk factors, this association became nonsignificant. Serum levels of retinol and α-tocopherol were nonsignificantly higher among case than control subjects.

In another large cross-sectional arterial imaging study, carotid artery IMT was determined by quartile of erythrocyte vitamin E and plasma carotenoid levels in 1187 men and women (ages 59–71 yr) who initially did not have CVD *(73)*. After adjustment for CVD risk factors, erythrocyte vitamin E levels were inversely and significantly associated with carotid artery IMT in men and women; plasma carotenoid levels were not associated with carotid IMT in either gender.

A longitudinal study examined the relationship of vitamin E and β-carotene plasma levels with the 12-mo progression of carotid IMT in 216 men without CVD who had

serum LDL-C levels greater than 4.01 mmol/L (>155 mg/dL) *(74)*. After adjusting for age, LDL-C level, systolic blood pressure, smoking, and other variables, there was an inverse correlation between progression of carotid artery IMT and vitamin E plasma levels ($p = 0.018$), as well as with β-carotene levels ($p = 0.012$). The adjusted mean 12-mo carotid IMT increase was 78% ($p = 0.045$) greater in the lowest (≤22.5 μmol/L) than in the highest (≥32.7 μmol/L) quartile of vitamin E plasma levels, and 92% greater ($p = 0.028$) in the lowest (≤0.27 μmol/L) than in the highest (≥0.64 μmol/L) quartile of β-carotene plasma levels.

Evidence demonstrating a beneficial effect of supplementary vitamin E intake on progression of early atherosclerosis was obtained from analysis of the CLAS carotid artery IMT data *(7)*. Subjects in the CLAS placebo group who ingested more than 100 IU/d of supplemental vitamin E demonstrated less progression of carotid IMT over a 2-yr period than those who ingested less than 100 IU/d (0.008 vs 0.023 mm/yr; $p = 0.02$). These data indicate that supplemental vitamin E intake may have a beneficial effect on reducing the progression of early preintrusive atherosclerosis. The lack of effect of supplemental vitamin E intake on the progression of carotid IMT in the drug group most likely reflects a dominating effect of lipid-lowering on the progression of early atherosclerosis . When examined independently of supplementary vitamin intake, dietary vitamin E and C intake had no beneficial effect on the progression of carotid IMT. Supplementary vitamin C intake had no beneficial effect on the progression of carotid IMT.

4. COMPLETED RANDOMIZED CONTROLLED TRIALS

Although the conclusions from epidemiological studies concerning the CVD protective effects of antioxidant vitamins have been mixed, there was enough consistency in the data and potential benefit in reducing disease risk to warrant clinical trials. Epidemiological studies are unable to completely control for confounding variables that could affect disease outcome. It is not possible to conclusively determine whether the association of CVD with dietary assessment or serum measurements of antioxidant vitamins represent a true association or are confounded by other dietary or lifestyle practices, which themselves are protective. Additionally, the protective effects seen in these studies may be a result of other components of the foods or a combination of antioxidants or these other food components. The epidemiological data examining the relationship between antioxidant vitamin intake and CVD are limited in providing definitive answers regarding whether antioxidant vitamins are protective for CVD. Unbiased estimates of the efficacy of antioxidant vitamins as therapeutic or preventive agents can only be obtained from RCTs.

To date, 19 RCTs of antioxidant vitamin supplementation examining CVD events and atherosclerosis progression have been published (Tables 2–4). Of these 19 RCTs, 8 were designed to determine whether antioxidant vitamin supplementation was effective in preventing cancer with CVD as a secondary outcome (Table 2; refs. *93–102*). These RCTs were conducted in individuals predominantly asymptomatic for CVD at baseline. The remaining 11 RCTs were specifically designed to study CVD outcomes with antioxidant vitamin supplementation (Tables 3 and 4). Of these 11 RCTs, 5 have been conducted in individuals with established CVD at baseline *(103–107)*, 1 has been conducted in individuals without established CVD (Table 3; ref. *108*), and 5 have been arterial imaging trials to measure the progression of atherosclerosis (Table 4; refs. *109–113*).

Table 2
Randomized Controlled Trials of Supplementary Vitamin Intake With Cardiovascular Disease as a Secondary Outcome

Vitamin E
Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC)
Linxian Study
Vitamin C
Linxian Study
Geriatric Hospital Study
β-Carotene
Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC)
Linxian study
Beta-Carotene and Retinol Efficacy Trial (CARET)
Skin Cancer Prevention Study (SCPS)
Physicians' Health Study (PHS)
Women's Health Study (WHS)
Nambour Skin Cancer Prevention Trial (NSCPS)

Table 3
Randomized Controlled Trials of Supplementary Vitamin Intake With Cardiovascular Disease as the Primary Outcome

Vitamin E
Secondary Prevention Trials
Cambridge Heart Antioxidant Study (CHAOS)
Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico Acuto Prevenzione Trial (GISSI)
Heart Outcomes Prevention Evaluation (HOPE) Study
Secondary Prevention with Antioxidants of Cardiovascular Disease in Endstage Renal Disease (SPACE)
Heart Protection Study (HPS)
Primary Prevention Trial
Primary Prevention Project (PPP)

4.1. Randomized Controlled Trials With CVD as a Secondary Outcome

4.1.1. Vitamin E

The Alpha-Tocopherol Beta-Carotene Cancer (ATBC) Prevention Study was a double-blind, placebo-controlled, primary prevention RCT designed to determine whether daily R,R,R-α-tocopherol, β-carotene, or both, would reduce the incidence of lung and other cancers *(93)*. A total of 29,133 male Finnish smokers (ages 50–69 yr) were randomized in a 2×2 factorial design to α-tocopherol (50 mg/d) alone, β-carotene (20 mg/d) alone, both α-tocopherol and β-carotene, or placebo. After a median follow-up of 6.1 yr of randomized treatment, there was no overall benefit of either supplement on CVD. Relative to the individuals who did not receive α-tocopherol, those assigned to α-tocopherol experienced a −16% (95% CI, −41 to 19%) nonsignificant reduction in death from ischemic stroke but a statistically significant 50% (95% CI, 2–120%) increase in death from hemorrhagic stroke. Individuals assigned to α-tocopherol had a −5% (95% CI, −15 to 6%) nonsignificant reduction in death from IHD but a 2% (95% CI, −5 to

Table 4
Arterial Imaging Randomized Controlled Trials

Coronary Angiographic Trials
HDL-Atherosclerosis Treatment Study (HATS)
Women's Angiographic Vitamin and Estrogen (WAVE) Trial
B-Mode Ultrasound Trials
Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) Study
Study to Evaluate Carotid Ultrasound Changes in Patients Treated with Ramipril and Vitamin E (SECURE)
Vitamin E Atherosclerosis Prevention Study (VEAPS)

9%) higher overall mortality that was also nonsignificant compared to those individuals who did not receive α-tocopherol. Regarding the primary trial end point, the β-carotene group experienced a statistically significant 18% (95% CI, 3–36%; $p = 0.01$) increase in the incidence of lung cancer relative to the placebo group. Subjects receiving α-tocopherol had a statistically significant –34% (95% CI, –49 to –15%) lower incidence of prostate cancer than control subjects; those receiving β-carotene had a 23% (95% CI, –4 to 58%) nonsignificantly greater incidence of prostate cancer than control subjects. Those receiving α-tocopherol also had a nonsignificant increase in bladder and stomach cancer incidence. The incidence of lung cancer was unaffected by α-tocopherol supplementation.

The first major coronary event after randomization was determined in the subset of 1862 men randomized to the ATBC Prevention Study who had a previous MI *(94)*. In this subset of subjects, 424 nonfatal MIs and fatal cases of CHD occurred during follow-up. Relative to subjects who received placebo (438 subjects), those assigned only to the α-tocopherol group (466 subjects) experienced a –38% (95% CI, –59 to –4%) significant reduction in nonfatal MI. However, there was a 33% (95% CI, –14 to 105%) nonsignificant increase in fatal cases of CHD in the α-tocopherol group. All events combined (nonfatal MI and fatal CHD) were nonsignificantly reduced by –10% (95% CI, –33 to 22%) in subjects assigned to the α-tocopherol group. Subjects assigned to the α-tocopherol plus β-carotene group experienced a 58% (95% CI, 5–140%) significant increase in death from CHD relative to the placebo group. Combined nonfatal MI and fatal CHD were nonsignificantly increased 14% (95% CI, –13 to 51%) in the α-tocopherol plus β-carotene group. Nonfatal MI was nonsignificantly reduced –14% (95% CI, –42 to 26%) in the α-tocopherol plus β-carotene group.

The first major coronary event after randomization also was determined in the subset of 27,271 men randomized to the ATBC Prevention Study who did not have a previous MI *(95)*. In this subset of subjects, 2111 nonfatal MIs and fatal cases of CHD occurred during follow-up. Relative to subjects who received placebo (6849 subjects), those assigned only to the α-tocopherol group (6820 subjects) experienced a 4% (95% CI, –11 to 22%) nonsignificant increase in nonfatal MIs. Fatal CHD was nonsignificantly decreased –10% (95% CI, –25 to 8%) in the α-tocopherol group. All events combined (nonfatal MI and fatal CHD) were nonsignificantly reduced by –2% (95% CI, –13 to 10%) in subjects assigned to the α-tocopherol group. Subjects assigned to the α-tocopherol plus β-carotene group experienced a –6% (95% –21 to 13%) nonsignificant decrease in death from CHD relative to the placebo group. All events combined (nonfatal MI and fatal CHD) were nonsignificantly decreased –3% (95% CI, –14 to 9%)

in the α-tocopherol plus β-carotene group. Nonfatal MI was nonsignificantly reduced by −1% (95% CI, −16 to 16%) in the α-tocopherol plus β-carotene group.

The Linxian Study was a randomized, double-blind, placebo-controlled, primary prevention trial designed to determine whether daily intake of four combinations of nine individual vitamin-mineral supplements (2 × 4 factorial design) would reduce overall or cancer mortality or incidence of cancer *(96)*. Subjects (ages 40–69 yr) were recruited from four Linxian (Chinese) communities, and 29,584 individuals were randomized to placebo or retinol (5000 IU) and zinc (22.5 mg); riboflavin (3.2 mg) and niacin (40 mg); vitamin C (120 mg) and molybdenum (30 μg); and vitamin E (30 mg), β-carotene (15 mg), and selenium (Se) (50 μg). Linxian Province was chosen because inhabitants of this area have one of the highest esophageal/gastric cancer rates in the world, as well as a low intake of several micronutrients. After a follow-up of 5.25 yr of randomized treatment, total mortality was significantly reduced (RR = −9%, 95% CI, −16 to −1%; $p = 0.03$) among those randomized to the vitamin E, β-carotene, Se supplementation group relative to placebo. This reduction mostly resulted from lower cancer mortality, especially stomach cancer. There was also a nonsignificant reduction in cerebrovascular mortality (RR = −10%, 95% CI, −24 to 7%). Because vitamin E, β-carotene, and Se were used in combination, it was not possible to determine which supplement or supplements contributed to the lower mortality rates. The other three combination regimens of retinol and zinc, riboflavin and niacin, and vitamin C and molybdenum had no significant effects on mortality rates. The relevance of these results to Western populations is unclear.

4.1.2. Vitamin C

In the Linxian study, there was no significant effect on mortality rates in the vitamin C plus molybdenum supplement group *(96)*. In a smaller, separate study of 538 subjects (ages 52–97 yr), there was no reduction in total mortality at 6 mo in those individuals randomized to 200 mg/d of vitamin C vs those taking placebo *(97)*.

4.1.3. β-Carotene

In the ATBC Prevention Study, subjects randomized to β-carotene experienced an 8% (95% CI, 1–16%) significant increase in total mortality and a 12% (95% CI, 1–25%) significant increase in death from IHD compared to those who did not receive β-carotene *(93)*. In addition, relative to those individuals who did not receive β-carotene, those assigned to β-carotene experienced a 23% (95% CI, −14 to 76%) nonsignificant increase in death from ischemic stroke and a 17% (95% CI, −20 to 70%) nonsignificant increase in death from hemorrhagic stroke. In addition to apparently increasing all of the CVD mortality endpoints, β-carotene also significantly increased the incidence of lung cancer and nonsignificantly increased prostate, colon and rectal, and stomach cancer.

In the ATBC Prevention Study subset *(94)* of men who had a previous MI, subjects assigned to the β-carotene group (461 subjects) experienced a 75% (95% CI, 16–164%) significant increase in death from CHD compared to placebo subjects. All events combined (nonfatal MI and fatal CHD) were nonsignificantly increased 11% (95% CI, −16 to 48%) in subjects assigned to the β-carotene group. In the β-carotene group, nonfatal MI was nonsignificantly reduced by −33% (95% CI, −56 to 2%) relative to the placebo group.

In the ATBC Prevention Study subset *(95)* of men who did not have a previous MI, subjects assigned only to the β-carotene group (6821 subjects) experienced a −1% (95% CI, −17 to 19%) nonsignificant reduction in death from CHD. All events combined

(nonfatal MI and fatal CHD) were nonsignificantly increased by 3% (95% CI, –9 to 16%) in subjects assigned to the β-carotene group. In the β-carotene group, nonfatal MI was nonsignificantly increased 6% (95% CI, –10 to 24%), relative to the placebo group.

In the Linxian study, β-carotene supplementation was used in combination with vitamin E and Se *(96)*. The results of this combination of supplements on total mortality, CVD mortality, and cancer are summarized in Subheading 4.1.1.

The Beta-Carotene and Retinol Efficacy Trial (CARET) was a double-blind, placebo-controlled, primary prevention RCT designed to determine whether daily treatment with a combined supplement containing β-carotene and vitamin A would reduce the incidence of lung cancer, the primary trial endpoint *(98)*. A total of 18,314 men and women (ages 45–74 yr) with a high risk for lung cancer from asbestos and/or smoking exposure were randomized to placebo or a combined supplement of β-carotene (30 mg/d) and vitamin A (25,000 IU/d) in the form of retinyl palmitate. After a mean follow-up of 4 yr of randomized treatment, CARET was terminated early because of a 28% (95% CI, 4–57%; $p = 0.02$) significantly increased risk for lung cancer in the combined supplement group compared with the placebo group. In the combined supplement group, overall mortality was significantly increased by 17% (95% CI, 3–33%; $p = 0.02$), and death from CVD was nonsignificantly increased by 26% (95% CI, –1 to 61%) compared with the placebo group.

The Skin Cancer Prevention Study (SCPS) was a randomized, double-blind, placebo-controlled, secondary prevention trial designed to determine the effect of daily β-carotene supplementation on all-cause mortality and mortality from CVD and cancer in subjects with at least one biopsy-proven basal cell or squamous cell skin cancer *(99)*. A total of 1188 men and 532 women (mean age: 63 yr) were randomized to placebo or β-carotene (50 mg/d) supplementation. After a median follow-up of 4.3 yr of randomized treatment, subjects randomized to β-carotene supplementation showed a 3% (95% CI, –18 to 30%) nonsignificant increase in all-cause mortality and a 16% (95% CI, –18 to 64%) nonsignificant increase in CVD mortality compared with the placebo group. In subgroup analyses, there was no evidence of a protective effect of β-carotene supplementation on mortality in subjects with initial plasma β-carotene levels below the median concentration or among subjects classified by smoking history.

The Physicians' Health Study was a randomized, double-blind, placebo-controlled, primary prevention trial designed to test whether aspirin and β-carotene supplementation could prevent cancer and CVD *(100)*. Using a 2 × 2 factorial design, 22,071 US male physicians (ages 40–84 yr) without a history of cancer (except nonmelanomatous skin cancer) and CVD were randomized to aspirin (325 mg/d) plus β-carotene placebo, β-carotene (50 mg every other day) plus aspirin placebo, both active agents, or both placebos. At the beginning of the study, 11% of the subjects were current smokers and 39% were former smokers. The aspirin portion of the study was terminated early because of a 44% statistically significant ($p < 0.001$) reduction in risk for a first MI *(114)*. After a mean follow-up of 12 yr of randomized treatment, subjects randomized to β-carotene supplementation showed no statistically significant overall benefit or harm with respect to cancer, cardiovascular events, CVD mortality, or total mortality *(100)*. However, there were certain trends consistent with the ATBC Prevention *(93–95)*, CARET *(98)*, and SCPS *(99)* studies in the current smokers who were randomized to β-carotene supplementation. Current smokers randomized to β-carotene supplementation had an 8% (95% CI, –20 to 48%) greater risk for MI, 18% (95% CI, –17 to 67%) greater risk for

stroke, 15% (95% CI, –7 to 43%) greater risk for cardiovascular events (nonfatal MIs, nonfatal strokes, and CVD deaths), 13% (95% CI, –20 to 61%) greater risk for CVD mortality, and 5% (95% CI, –14 to 29%) greater risk for death from all causes compared with placebo subjects; all relative risks were nonsignificant *(100)*.

The Women's Health Study was a randomized, double-blind, placebo-controlled trial using a 2 × 2 × 2 factorial design to test aspirin (100 mg every other day), vitamin E (600 IU every other day), and β-carotene (50 mg every other day) in the prevention of cancer and CVD in 39,876 women ages 45 yr or older *(101)*. The β-carotene component was terminated early after a median intervention of 2.1 yr primarily because of the null findings on β-carotene and cancer incidence after 12 yr of randomized treatment in the companion Physicians' Health Study. Compared with placebo, there were no statistically significant differences in the incidence of cancer, CVD, or total mortality after the 2.1-yr treatment period or after an additional 2-yr follow-up. MI was nonsignificantly reduced by –16% (95% CI, –44 to 27%) in the β-carotene group relative to placebo group, whereas stroke was nonsignificantly increased by 42% (95% CI, –4 to 110%), and death from CVD nonsignificantly increased 17% (95% CI, –46 to 153%) in the β-carotene group relative to the placebo group.

The Nambour Skin Cancer Prevention Trial (NSCPT) was a randomized, double-blind, placebo-controlled trial using a 2 × 2 factorial design to test β-carotene (30 mg/d) and 15-plus sunscreen in the prevention of basal and squamous cell carcinomas in 1621 residents (ages 20–69 yr) of Nambour in southeast Queensland, Australia *(102)*. After 4.5 yr of intervention, there were no significant differences in the incidence of new skin cancers between the treatment groups. During the course of the trial, there were 11 deaths among the 801 participants randomized to β-carotene supplementation and 21 deaths among the 820 participants randomized to placebo, resulting in a nonsignificant –50% (95% CI, –76 to 3%) reduction in overall mortality. Although there were few CVD deaths (6 in the β-carotene group and 12 in the placebo group), they were nonsignificantly reduced by 50%.

4.2. Randomized Controlled Trials With CVD as the Primary Outcome

4.2.1. Secondary Prevention Trials

The Cambridge Heart Antioxidant Study (CHAOS) was a randomized, double-blind, placebo-controlled, secondary prevention trial designed to determine whether α-tocopherol would reduce CVD risk *(103)*. The primary trial outcomes were a combined endpoint of CVD death and nonfatal MI and nonfatal MI alone. A total of 2002 subjects with angiographically proven CAD were randomized to α-tocopherol (1035 subjects) and placebo (967 subjects). The first 546 subjects assigned to the α-tocopherol group received 800 IU/d; the remainder received 400 IU/d. After a median follow-up of 510 d, subjects assigned to the α-tocopherol group experienced a significant reduction of –47% (95% CI, –66 to –17%; $p = 0.005$) in the CVD death and nonfatal MI combined endpoint. The beneficial effect on the composite endpoint resulted from a –77% (95% CI, –89 to –53%; $p = 0.005$) significant reduction in the risk for nonfatal MI. However, there was a nonsignificant increase 18% (95% CI, –38 to 127%) for CVD death in the α-tocopherol group. In addition, total mortality was nonsignificantly greater in the α-tocopherol group than in the placebo group (3.5 vs 2.7%; $p = 0.31$). The pattern of a significant reduction in nonfatal MI and a nonsignificant increase in CVD death in CHAOS is similar to the results from the ATBC substudy and remains unexplained *(94)*.

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI) Prevenzione trial was an open-label trial conducted in 1665 women and 9659 men (average age: 59.4 yr) who had a recent (≤3 mo) MI *(104)*. Subjects were randomly assigned according to a 2 × 2 factorial design to receive either 300 mg of synthetic vitamin E daily or no supplement and either an n-3 polyunsaturated fatty acid (PUFA) supplement or no supplement for a mean of 3.5 yr. The primary trial outcome was a composite of death, nonfatal MI, and stroke. Intention-to-treat analyses were done according to the factorial design (two-way) and by treatment group (four-way). Vitamin E had no beneficial effect in either analysis on the primary trial endpoint relative to the other treatment groups. By the two-way analysis, a total of 730 of the 5660 subjects randomized to vitamin E (12.9%) and 770 of the 5664 subjects randomized to the control group (13.6%) had a primary outcome event, yielding a nonsignificantly reduced risk of −5% (95% CI, −14 to 5%). Cardiovascular death was also nonsignificantly decreased by −6% (95% CI, −19 to 10%). By the four-way analysis, a total of 371 of the 2830 subjects randomized to vitamin E alone (13.1%) and 414 of the 2828 subjects randomized to the control group (14.6%) had a primary outcome event, yielding a nonsignificantly reduced risk of −11% (95% CI, −23 to 3%). Cardiovascular death was significantly decreased −20% (95% CI, −35 to −1%) in the four-way analysis. On the other hand, both the two-way and the four-way analyses indicated a significant reduction of −10 to −15% (95% CI, −26 to −1%) in the primary trial outcome and a significant reduction of −17 to −30% (95% CI, −29 to −3%) in cardiovascular death by n-3 PUFA relative to the other treatment groups.

The Heart Outcomes Prevention Evaluation (HOPE) Study was a double-blind, placebo-controlled trial conducted in 2545 women and 6996 men (ages 55 yr or older) who had either established CVD or diabetes in addition to one other CVD risk factor *(105)*. Subjects were randomly assigned according to a 2 × 2 factorial design to receive either 400 IU of natural vitamin E daily or matching placebo and either Ramipril (an angiotensin-converting enzyme inhibitor) or matching placebo for a mean of 4.5 yr. The primary trial outcome was a composite of MI, stroke, and death from cardiovascular causes. A total of 772 (16.2%) of the 4761 subjects randomized to vitamin E and 739 (15.5%) of the 4780 subjects randomized to placebo had a primary outcome event (RR = 5%, 95% CI, −5 to 16%; $p = 0.33$). There were also no significant differences between vitamin E and placebo in the components of the composite primary trial outcome with a nonsignificant 17% (95% CI, −5 to 42%; $p = 0.13$) increase in stroke and a 5% (95% CI, −10 to 22%; $p = 0.54$) increase in cardiovascular death. On the other hand, relative to subjects randomized to placebo, subjects receiving Ramipril had a significant reduction of −22% (95% CI, −30 to −14%; $p < 0.001$) in the primary trial outcome *(115)*.

The Secondary Prevention with Antioxidants of Cardiovascular Disease in Endstage Renal Disease (SPACE) was a double-blind, placebo-controlled trial conducted in 61 women and 135 men (ages 40–75 yr) who had pre-existing CVD *(106)*. All participants were chronic hemodialysis patients. Subjects were randomly assigned to either 800 IU of natural vitamin E daily or matching placebo for a median of 519 d. The primary trial outcome was a composite of fatal and nonfatal MI, stroke, peripheral vascular disease, and unstable angina. Fifteen (16%) of the 97 subjects randomized to vitamin E and 33 (33%) of the 99 subjects randomized to placebo had a primary outcome event, a significant reduction in risk of −54% (95% CI, −73 to −22%; $p = 0.014$). Five (5.1%) of the 97 subjects randomized to vitamin E and 17 (17.2%) of the 99 subjects randomized to placebo had a MI, a significant

reduction in risk of −70% (95% CI, −89 to −22%; $p = 0.016$). CVD mortality was nonsignificantly reduced by −39% (95% CI, −72 to 30%).

The Heart Protection Study was a double-blind, placebo-controlled trial conducted in 5082 women and 15,454 men (ages 40–80 yr) who had either established CAD, other occlusive vascular disease, or diabetes *(107)*. Subjects were randomly assigned according to a 2 × 2 factorial design to receive either antioxidant vitamin supplementation (600 mg of synthetic vitamin E, 250 mg of vitamin C, and 20 mg of β-carotene daily) or matching placebo and either simvastatin or matching placebo for a mean of 5 yr. The primary outcomes were fatal and nonfatal vascular events. There was no significant difference in all-cause mortality between subjects randomized to antioxidant vitamin supplementation (1446 of 10,269 subjects [14.1%]) and subjects randomized to placebo (1389 of 10,267 subjects [13.5%]), for an increased risk of 4% (95% CI, −3 to 12%; $p = 0.30$). Similarly, there were no significant differences in deaths resulting from vascular or nonvascular causes. A total of 878 (8.6%) of the 10,269 subjects randomized to antioxidant vitamin supplementation and 840 (8.2%) of the 10,267 subjects randomized to placebo died from a vascular cause, for an increased risk of 5% (95% CI, −5 to 15%; $p = 0.30$). There was no significant difference in the number of subjects having a nonfatal MI or coronary death: 1063 (10.4%) of 10,269 subjects randomized to antioxidant vitamin supplementation vs 1047 (10.2%) of 10,267 subjects randomized to placebo, for an increased risk of 2% (95% CI, −6 to 11%; $p = 0.70$). There was no significant difference in the number of subjects who had a fatal or nonfatal stroke, 511 (5%) of 10,269 subjects randomized to antioxidant vitamin supplementation vs 518 (5%) of 10,267 subjects randomized to placebo, for a decreased risk of −1% (95% CI, −13 to 12%; $p = 0.80$). On the other hand, relative to subjects randomized to placebo, subjects receiving simvastatin had a significant reduction of −13% (95% CI, −9 to −6%; $p = 0.0003$) in all-cause mortality and a −17 to −27% significant reduction in cardiovascular mortality and vascular events *(116)*.

4.2.2. Primary Prevention Trials

The Primary Prevention Project was an open-label trial conducted in 2583 women and 1912 men (mean age: 64.4 yr) without pre-existing CVD *(108)*. Subjects were randomly assigned according to a 2 × 2 factorial design to receive either 300 mg of synthetic vitamin E daily or no supplement and either low-dose aspirin or no aspirin for a mean of 3.6 yr. The primary trial outcome was a composite of cardiovascular death, nonfatal MI, stroke, and nonfatal stroke. A total of 56 (2.5%) of the 2231 subjects randomized to vitamin E and 53 (2.3%) of the 2264 subjects randomized to placebo had a primary outcome event, yielding a nonsignificant increased risk of 7% (95% CI, −26 to 56%). There were also no significant differences between vitamin E and no vitamin E in the components of the composite primary trial outcome, with a nonsignificant reduction of −14% (95% CI, −51 to 52%) in cardiovascular death, an increase of 1% (95% CI, −44 to 103%) in nonfatal MI and an increase of 56% (95% CI, −44 to 103%) in nonfatal stroke. On the other hand, subjects receiving low-dose aspirin had a significant reduction of −24% (95% CI, −69 to −1%) in cardiovascular death relative to subjects not receiving aspirin.

4.2.3. Arterial Imaging Trials

4.2.3.1. Coronary Angiographical Trials. The HDL-Atherosclerosis Treatment Study (HATS) was a randomized, double-blind, placebo-controlled trial of 160 subjects (13% female; average age: 53 yr) with CAD and a low HDL-cholesterol level (≤35 mg/dL

for men, ≤40 mg/dL for women) *(109)*. Subjects with angiographically documented CAD were randomly assigned to one of four regimens: antioxidants (800 IU of synthetic vitamin E, 1000 mg of vitamin C, 25 mg of natural β-carotene, and 100 μg of Se daily), simvastatin-niacin plus antioxidants, simvastatin-niacin, or placebos. After 3 yr of intervention, standardized coronary angiograms were performed and compared with the standardized baseline coronary angiograms. The primary trial endpoints were angiographic evidence of a change in coronary stenosis and the occurrence of a cardiovascular event, death, MI, stroke, or revascularization. The simvastatin and niacin dosages were titrated to obtain an LDL-cholesterol goal of less than 90 mg/dL and to raise HDL-cholesterol by at least 10 mg/dL over baseline. The mean per-subject change in lesion %S was +3.9%S with placebos, –0.4%S with simvastatin-niacin alone ($p < 0.001$ for the comparison with the placebo group), +1.8%S with antioxidants alone ($p = 0.16$ for the comparison with the placebo group), and +0.7%S with simvastatin-niacin plus antioxidants ($p = 0.004$ for the comparison with the placebo group). The frequency of clinical event endpoints was 24% in the placebo group, 3% in the simvastatin-niacin group, 21% in the antioxidant group, and 14% in the simvastatin-niacin plus antioxidant group. Only the frequency of clinical events in the simvastatin-niacin group significantly ($p = 0.003$) differed from the placebo group.

The Women's Angiographic Vitamin and Estrogen (WAVE) Trial was a double-blind, placebo-controlled trial conducted in 423 postmenopausal women (average age: 65 yr) with angiographically established CAD *(110)*. Subjects were randomly assigned according to a 2 × 2 factorial design to receive either 400 IU of vitamin E plus 500 mg of vitamin C daily or matching placebo and either 0.625 mg of conjugated equine estrogen daily (plus 2.5 mg of medroxyprogesterone acetate daily for women who had not had a hysterectomy) or matching placebo. The mean interval between the baseline and follow-up coronary angiograms was 2.8 yr. The primary clinical trial endpoint was based on the change in the mean minimum lumen diameter of all qualifying coronary arterial segments and on the incidence of MI and death. The mean minimum lumen diameter (in millimeters/year [mm/yr]) worsened in all four groups: –0.0010 mm/yr with placebos, –0.048 mm/yr with hormone therapy alone, –0.042 mm/yr with antioxidant therapy alone, and –0.046 mm/yr with antioxidant therapy plus hormone therapy; none of the differences compared with placebo were significant. The frequency of the composite clinical endpoint was 2.8% in the placebo group, 4.9% in the hormone therapy group, 6.7% in the antioxidant group, and 6.5% in the antioxidant therapy plus hormone therapy group. The frequency of clinical events was not significantly different among groups ($p = 0.54$). However, all-cause mortality was significantly higher in women assigned to antioxidant vitamin supplementation compared with women assigned to antioxidant vitamin placebo (16 vs 6; hazard ratio = 2.6; 95% CI, 1.1–7.2; $p = 0.047$).

4.2.3.2. B-Mode Ultrasound Trials. The Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) Study was a double-blind, placebo-controlled trial conducted in 264 postmenopausal women and 256 men (ages 45–69 yr) with serum cholesterol of no less than 194 mg/dL *(111)*. Subjects were randomly assigned according to a 2 × 2 factorial design to receive either 136 IU of vitamin E, 250 mg of slow-release vitamin C, a combination of both vitamins, or placebo twice daily for 3 yr. The primary trial endpoint was progression of common carotid artery IMT determined every 6 mo from serial high-resolution B-mode ultrasonograms. In women, there were no significant

differences in progression rates among the treatment groups: 0.016 mm/yr in women randomized to placebo, 0.015 mm/yr in women who received vitamin E, 0.017 mm/yr in women who received only vitamin C, and 0.016 mm/yr in women who received the vitamin combination. In men, the rates of progression were 0.020 mm/yr in those randomized to placebo, 0.018 mm/yr in those who received only vitamin E, 0.017 mm/yr in those who received only vitamin C, and 0.011 mm/yr in those who received both vitamins. The progression rate was significantly less in the men who were randomized to both vitamins compared to all men ($p = 0.009$) or compared with men who received placebo ($p = 0.008$). The treatment effect of the vitamin combination was limited to hypercholesterolemic men who smoked. Baseline plasma α-tocopherol and total ascorbate levels were lowest among men who smoked relative to men who did not smoke or to women who did or did not smoke.

The Study to Evaluate Carotid Ultrasound Changes in Patients Treated with Ramipril and Vitamin E (SECURE) *(112)* was a substudy of HOPE *(105)*. SECURE was a double-blind, placebo-controlled trial conducted in 172 women and 560 men (ages 55 yr or older) who had either established CVD or diabetes in addition to one other cardiovascular risk factor. Subjects were randomly assigned according to a 3×2 factorial design to receive either 400 IU of natural vitamin E daily or matching placebo and either 2.5 or 10 mg/d of Ramipril or matching placebo for a mean of 4.5 yr. The primary trial endpoint was progression of the mean maximum carotid artery intima-media thickness determined every 6 mo from serial high-resolution B-mode ultrasonograms. There were no differences in progression rates between patients randomized to vitamin E and those randomized to placebo (0.0174 vs 0.0180 mm/yr, respectively). On the other hand, the progression rates among patients randomized to Ramipril were significantly less than the progression rate of patients randomized to placebo (0.021 mm/yr for the placebo group, 0.0180 mm/yr for the Ramipril group receiving 2.5 mg/d, and 0.0137 mm/yr for the Ramipril group receiving 10 mg/d; $p = 0.033$).

The Vitamin E Atherosclerosis Prevention Study (VEAPS) was a randomized, double-blind, placebo-controlled trial conducted in 185 women and 168 men (age 40 yr or older) with LDL-cholesterol 130 mg/dL or higher and no clinical signs or symptoms of CVD *(113)*. Subjects were randomly assigned to either 400 IU of synthetic vitamin E daily or matching placebo for 3 yr. The primary trial endpoint was progression of common carotid artery IMT, a well-validated measure of subclinical atherosclerosis *(89–91)* determined every 6 mo from serial high-resolution B-mode ultrasonograms. Although on-trial plasma vitamin E levels were significantly increased and several markers indicated a significant reduction in LDL oxidation in the vitamin E group, there was no difference in the average rates of atherosclerosis progression between the two treatment groups. In fact, the rate of progression in the vitamin E group was twice that of the placebo group (0.0040 vs 0.0023 mm/yr; $p = 0.08$).

5. SUMMARY OF RANDOMIZED CONTROLLED TRIALS OF ANTIOXIDANT VITAMIN SUPPLEMENTATION

Publication of no less than 19 RCTs examining the effects of antioxidant vitamin supplements on CVD events and atherosclerosis progression have yielded inconsistent, conflicting, and, occasionally, somewhat troublesome data. In general, when one examines the vitamin E data (Tables 2–4), there is a general null effect of this antioxidant supplement on CVD events and on the progression of atherosclerosis . In fact, when one examines the data

carefully, there are some troublesome results. In ATBC, there was no overall benefit of vitamin E supplementation on CVD. However, subjects who received vitamin E had a significantly greater risk of death from hemorrhagic stroke *(93)*. In CHAOS, although there was a reduction in risk from nonfatal MI, there was a nonsignificant increased risk for both CVD death and total mortality in the vitamin E group *(103)*. Similar to the CHAOS study, the ATBC substudy *(94)* in men with previous MI showed the same pattern of a significant reduction in nonfatal MI but a nonsignificant increase in CVD death. Interestingly, the increase in risk for CVD death was not apparent in the ATBC substudy of men without a previous MI *(95)*. The cohort of ATBC was comprised of individuals who smoked *(93)*. In the arterial imaging trials, VEAPS *(113)* showed a nonsignificant greater progression rate of subclinical atherosclerosis in healthy men and women receiving vitamin E relative to placebo, whereas ASAP *(111)* and SECURE *(112)* showed no effect of vitamin E supplementation relative to placebo. However, there have been trial results indicating a significant reduction of CVD events with vitamin E supplementation, as in CHAOS *(103)* and the smaller SPACE trial *(106)*. CHAOS and SPACE are similar in that both of these trials used a higher dosage of vitamin E (in CHAOS, a lower dosage was subsequently used) relative to most of the other trials, and they were conducted for the shortest time periods relative to other trials. SPACE, like CHAOS, did not show a reduction in CVD mortality with vitamin E supplementation. The only trial that showed a significant reduction in CVD mortality was GISSI *(104)*. However, this finding was restricted to the four-way analysis. Neither the two-way nor the four-way analysis showed a significant reduction in the primary trial outcome composite of death, nonfatal MI, and stroke.

In the two randomized controlled trials that studied vitamin C alone *(96,97)*, there was no evidence that vitamin C was effective in reducing CVD events or mortality. No adverse effects were observed.

The results from the ATBC *(93–95)*, Linxian *(96)*, CARET *(98)*, SCPS *(99)*, Physicians' Health Study *(100)*, Women's Health Study *(101)*, and NSCPT *(102)* trials provided no support for a beneficial effect of β-carotene in the prevention of CVD in contradistinction to the beneficial effects inferred from observational studies. In fact, taken together, the results of these trials are troubling because subjects, particularly smokers randomized to β-carotene supplementation groups, had increased rates of cancer, cardiovascular events, CVD mortality, and overall mortality. In particular, in the ATBC substudy of subjects who had a previous MI, those randomized to β-carotene supplementation had a 75% significantly greater risk of CVD mortality compared with placebo *(94)*. Those subjects who received both vitamin E and β-carotene supplementation had a 58% significantly greater risk of CVD mortality than those receiving placebo *(94)*. These trials indicate that β-carotene is not the primary component responsible for the lower risks of cancer and CVD associated with the high intakes of fruits and vegetables. Although one could argue that β-carotene should still be tested in other populations, there is little support for efficacy and good reason for concern for the safety of supplemental β-carotene for the prevention of cancer and CVD in certain populations, such as smokers.

The serial arterial imaging trials provided important information regarding the effects of antioxidant supplements on the progression of atherosclerosis . The coronary angiographic trials HATS *(109)* and WAVE *(110)* showed that cocktails of antioxidants including vitamin E, vitamin C, β-carotene, and Se have no beneficial effect on the progression of established coronary artery atherosclerosis . In fact, in HATS, when antioxidant therapy was combined with simvastatin-niacin, the coronary angiographic and clinical event benefits of simvastatin-niacin alone were diminished. In terms of the

angiographic endpoint, this adverse interaction between the antioxidant and the simvastatin-niacin therapies was significant ($p = 0.02$). This interaction appeared to be the result of a significant blunting of the increase in the HDL_2 atherosclerosis protective subfraction by the antioxidant therapy *(109)*.

In contrast to coronary angiography, which is a measurement endpoint for established late-stage atherosclerosis, B-mode carotid artery ultrasound is used to measure arterial wall thickness, the earliest anatomical manifestation of atherosclerosis *(117)*. Three completed trials, VEAPS *(113)*, SECURE *(112)*, and ASAP *(111)*, showed that vitamin E has no effect on the progression of subclinical atherosclerosis. In fact, in VEAPS, subjects who were randomized to vitamin E had a nonsignificant two-fold greater progression rate of subclinical atherosclerosis than those subjects randomized to placebo. This effect was seen in men and women. ASAP also showed that vitamin C alone had no effect on the progression of subclinical atherosclerosis *(111)*. In contrast to the null effects of vitamin E and C alone, ASAP showed that a combination of vitamin E and vitamin C had a significant effect in reducing the progression of subclinical atherosclerosis *(111)*. However, this effect was limited to males who smoked and had high serum cholesterol levels. Relative to the other groups of subjects in ASAP (nonsmoking men and women and smoking women), smoking men had low baseline serum vitamin C and E levels. Along with other trial results, such as those from SPACE *(106)*, ASAP indicated that individuals who are vitamin deficient may derive anti-atherosclerosis benefits from antioxidant vitamin supplementation. Although serum vitamin levels were not reported in SPACE, it is generally believed that chronic dialysis patients have low intracellular vitamin concentrations and experience a much greater level of oxidative stress than most other populations studied, with the possible exception of smokers.

It remains to be seen whether administration of antioxidant vitamin supplementation in relation to earliest stages of atherosclerosis (perhaps during childhood or young adulthood) will be effective in reducing progression. By the time atherosclerosis is well-established, it appears that antioxidant vitamin supplementation is ineffective in reducing progression and clinical coronary events. Animal studies have predominantly demonstrated antioxidants to be effective in preventing atherosclerosis, usually when it is in its earliest stages. There are very little data indicating that antioxidant vitamin supplementation may be therapeutically effective in treating atherosclerosis once it is established.

Epidemiological studies probably reflect long-term lifestyle behaviors, such as high dietary and supplementary intake of vitamins over many years, perhaps even during the early formative years of atherosclerosis development. In contrast, the RCTs have investigated vitamin intervention for a limited period of time and, in most instances, with populations that already have some degree of atherosclerosis . Although inverse associations with vitamin intake found in epidemiological studies may have resulted from uncontrolled confounding, the divergent results of the observational and clinical trial data alternatively suggest that antioxidant vitamins may have different effects in preventing atherosclerosis, reducing atherosclerosis progression, or affecting the rupture of atherosclerosis plaques. Before final conclusions are reached regarding the effects of antioxidant vitamin supplements on CVD, questions raised by the completed randomized trials need to be addressed.

6. ONGOING RANDOMIZED CONTROLLED TRIALS

Several ongoing RCTs are still underway (Table 5). These trials are testing antioxidant vitamin supplements in both primary and secondary prevention of CVD. SU.VI.MAX is

Ongoing Randomized, Double-Blind, Placebo-Controlled Trials Testing Antioxidants Alone or in Combination With Other Agents in the Primary and Secondary Prevention of CVD

Trial	*Cohort*	*Agent and dosage*	*Primary end point*
Primary prevention			
PHS	22,000 U.S. males	Vitamin E (400 IU qod[a]) Vitamin C (500 mg/d) β-carotene (50 mg qod) Multivitamin (standard dose) (factorial design)	MI, stroke, and CVD mortality
WHS	40,000 U.S. women	Vitamin E (600 IU qod) + aspirin (100 mg qod)	MI, stroke, and CVD mortality
SU.VI.MAX	15,000 French men and women	Vitamin E (30 IU/d) + β-carotene (6 mg/d) + vitamin C (120 mg/d) + Se (100 μg/d) + zinc (20 mg/d)	MI, stroke, and CVD mortality
Secondary prevention			
WACS[b]	8000 U.S. women	Vitamin E (600 IU qod), β-carotene (50 mg qod[c]), vitamin C (500 mg qod), (factorial design)	MI, stroke, CA revascularization and CVD mortality
Arterial imaging			
FATS	60 men with CAD	Vitamin E (400 IU BID) + vitamin C (500 mg BID) + β-carotene (12.5 mg BID) + Se (50 μg BID) + triple lipid-lowering medication	Progression of CAD by quantitative coronary angiography
SMARTFED	150 men and women with diabetes and dyslipidemia	Vitamin E (400 IU/d)	Progression of CAD by quantitative coronary angiography
MCBIT	600 men and women with CA bypass surgery	Vitamin E (800 IU/d) + lovastatin (±LDL-pheresis)	Progression of CAD by quantitative coronary angiography

[a]qod = every other day.
[b]Prior CVD or three or more coronary risk factors.
[c]β-carotene was replaced with folate.
PHS, Physicians' Health Study; WHS, Women's Health Study; SU.VI.MAX, Supplementation en Vitamines et Mineraux Antioxidants trial; WACS, Women's Antioxidant Cardiovascular Study; FATS, Familial Atherosclerosis Treatment Study—Long-Term Follow-Up; SMARTFED, St. Michael's Atherosclerosis Regression Trial with Fenofibrate and Vitamin E in Diabetics; MCBIT, Munich Coronary Bypass Intervention Trial.

a large primary prevention trial that will hopefully provide important information about this population *(118)*. More secondary prevention trials have been conducted than primary prevention trials; therefore, more data are needed in this latter population.

7. RECOMMENDATIONS

Although observational data suggest that antioxidant vitamins may reduce CVD risk and basic research provides plausible mechanisms for such an effect, these data have not been substantiated by completed RCTs. Additionally, results from these completed RCTs raise a level of concern for the safety of certain antioxidant vitamin supplementation among certain subgroups. In the final analysis, current data do not support the use of antioxidant vitamin supplements for the prevention or treatment of CVD *(119)*. Guidelines for the use of antioxidant vitamin supplements for the prevention or treatment of CVD will have to await the results of ongoing RCTs and assessment of the effects of antioxidant vitamins in certain populations, such as those with low serum vitamin levels and those with chronic disease states that may take a toll on the body's antioxidant capacity (e.g., diabetics and other special populations).

A conclusion that is emerging, but clearly not fixed, at this point is that supplementation with specific antioxidant vitamins does not prevent development of CVD in a generally healthy population. However, special populations may represent a type of deficiency whose basis is a disease state that creates an excess oxidant burden. Under such circumstances, vitamin E or other specific antioxidants may provide a significant margin of protection. Based on the understanding of these limitations, current and future trials may be able to identify at-risk populations that can benefit from antioxidant vitamin supplementation. The recent paper on the European perspective on vitamin E regarding our current level of knowledge emphasized the importance of considering the nonantioxidant functions of vitamin E *(120)*. Novel mechanisms for the antiatherosclerotic properties of vitamin E—which involve modulation of cellular signaling, transcriptional regulation, induction of apoptosis and interaction regulatory pathways involved in drug metabolism—were used as examples to suggest that inconclusive or troubling findings from clinical trials may reflect potentially disturbing effects of vitamin E when inappropriately applied to a healthy population. Regardless of the results of these trials, however, consumption of fruits and vegetables high in antioxidant vitamins appears to be an important component of a healthy dietary intake and lifestyle.

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11 Health Effects of *Trans* Fatty Acids

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and Susanne H. F. Vermunt

KEY POINTS

- *Trans* fatty acids are found in hydrogenated fats and in fats from ruminants.
- The intake of *trans* fatty acids is declining in the United States and is approx 2% of energy.
- Tissue levels of *trans* fatty acids reflect dietary intakes.
- *Trans* fatty acids have an adverse effect on the serum lipoprotein profile.
- Epidemiological studies suggest a positive relationship between the intake of *trans* fatty acids and cardiovascular risk.
- There is no strong evidence that *trans* fatty acids increase cancer risk.
- Keep the intake of *trans* fatty acids as low as possible.

1. INTRODUCTION

The position of the double bond in the carbon chain of a fatty acid is indicated in several ways. When counted from the carboxyl end (–COOH) of the molecule, the position (x) is denoted by the 'Δ–x' nomenclature, whereas the 'n-x' classification is used when counting starts from the methyl end ($–CH_3$). Thus, 'n-3' means that the double bond is located at the third carbon atom from the methyl end. These double bonds can have either the *cis* or the *trans* configuration. *Cis* means that the two carbon atoms adjacent to the double bond point in the same direction, whereas in the *trans* configuration, the two carbon atoms are located at opposite sides of the double bond. As an example, α-linolenic acid (C18:3 n-3), which belongs to the n-3 family, and one of its *trans*-isomers (C18:3n-3 Δ–9c, 12c, 15tr) are shown in Fig. 1. These two molecules are so-called "geometrical isomers."

Trans fatty acids in the diet are derived from several sources. First, they are formed, to small extents, from polyunsaturated fatty acids (PUFAs) by bacteria in the first stomach (rumen) of ruminant animals.

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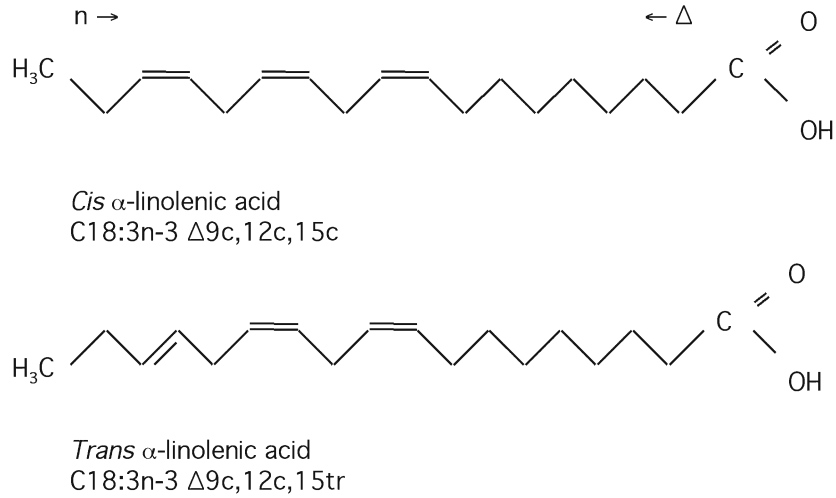

Fig. 1. Chemical structure of all-*cis* C18:3 n-3, and 9-*cis*, 12-*cis*, 15-*trans* C18:3 n-3 (C18:3 n-3 Δ9c, 12c, 15tr).

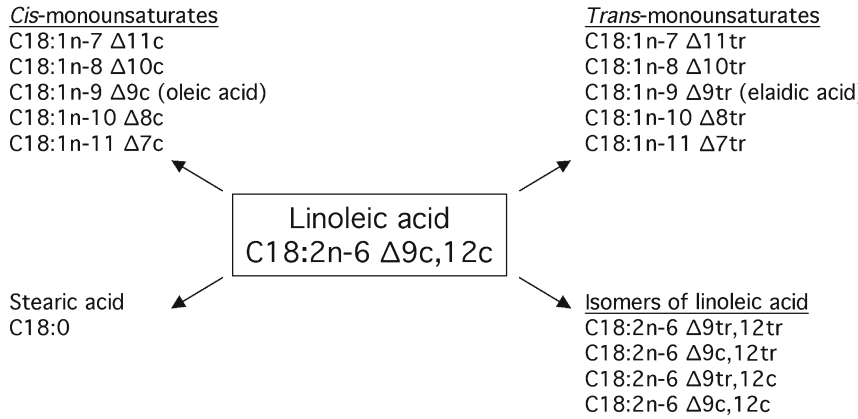

Fig. 2. Potential conversions of linoleic acid into its positional and geometrical isomers.

Second, *trans* fatty acids are produced by industrial hydrogenation and deodorization of vegetable oils. Hydrogenation is used to change the chemical, physical, and sensory characteristics of a vegetable oil to make the oil suitable for the production of foods such as margarines, shortenings, and biscuits. When all double bonds are hydrogenated, a saturated fatty acid is formed. However, the *cis* double bond may also isomerize into a *trans* double bond without net uptake of hydrogen. Furthermore, double bonds can migrate along the molecule, which results in the formation of positional isomers. Therefore, through hydrogenation, many different molecules can be formed (Fig. 2), although *trans* monounsaturated fatty acids are the most prevailing molecules.

Trans PUFAs—in particular those from α-linolenic acid—can be produced during deodorization, the last step of the refining process of vegetable oils. Finally, heating and frying of oils at high temperatures may also result in the formation of *trans* polyunsaturated bonds.

The beneficial effects of *cis* fatty acids on health differ from those of *trans* fatty acids. This chapter discusses the effects of *trans* fatty acids on various risk parameters for coronary heart disease (CHD), such as lipids and lipoproteins, low-density lipoprotein (LDL) oxidation, and hemostasis, and the effects of *trans* fatty acids on risks for cancer. The metabolism of *trans* fatty acids and their effects on the desaturation and elongation of other fatty acids is also addressed.

2. *TRANS* FATTY ACIDS IN FOODS

Trans fatty acids in margarines and dairy products are mainly found in triacylglycerols; in meat, *trans* fatty acids are found in phospholipids. Recent calculations suggest that the intake of *trans* fatty acids in the United States is declining. From 1980 to 1982, it was estimated that daily intakes of *trans* fatty acids were 3.0 and 2.8% of energy (8.4 and 5.4 g) in men and in women, respectively. During 1995–1997, these values were 2.2% for both men and women (6.4 and 4.7 g, respectively) *(1)*. For comparison, the intakes of saturated and monounsaturated fatty acids in 1995–1997 were about 11 to 12%. In Europe, the daily total *trans* fatty acid intake varied between 0.5% of energy in Italy to 2% of energy in Iceland *(2)*. Major contributors to total *trans* fatty acid intake are fried foods and snacks, stick margarines, baked goods, and, to a minor extent, dairy products. About 85% of all *trans* fatty acids in the diet are *trans* monounsaturated fatty acids, mostly C18:1 *trans*. When derived from ruminant fats, the double bond is mainly at the Δ−11 position (C18:1 Δ−11 *trans* or vaccenic acid). This is because these *trans*-isomers are formed through the specific action of bacteria in the rumen. In industrially hydrogenated oils, *trans*-isomers are formed chemically and, therefore, the double bond varies more or less randomly between positions Δ−8 and −13. The remaining 15% of *trans* fatty acid intake is provided primarily by mono-*trans*-isomers of linoleic and α-linolenic acid; a few di-*trans*-isomers also are present.

3. METABOLISM

3.1. Digestion, Absorption, and Incorporation into Blood Lipids

The metabolic pathways of *trans* fatty acids after intake are shown in Fig. 3. After ingestion, triacylglycerols are hydrolyzed by pancreatic lipase into free fatty acids, sn2-monoglycerides, and, to a small extent, diglycerides. Phospholipids are split by pancreatic phospholipases, which remove the fatty acid from the sn1 or sn2 position. As a result, lysophospholipids and free fatty acids are formed.

The breakdown products of triacylglycerol and phospholipids are incorporated into micelles and absorbed by the enterocyte. Hydrolysis and absorption of oleic acid and its positional and geometrical isomers are comparable. Within the enterocyte, the free fatty acids are used for the formation of triacylglycerols, cholesteryl esters, and phospholipids. After esterification, lipids are used for the formation of chylomicrons, which transport the fatty acids through the lymph to the liver. In the liver, fatty acids can be incorporated into very low-density lipoproteins (VLDLs). The incorporation of *trans*-isomers of C18:1 Δ−9 into VLDL, but also into other lipoproteins like LDL and high-density lipoproteins (HDLs), is lower than that of C18:1 Δ−9c. As any other fatty acid, *trans* fatty acids are also incorporated into tissue phospholipids, stored in adipose tissue, desaturated and elongated into longer chain PUFAs, and oxidized *(3–7)*.

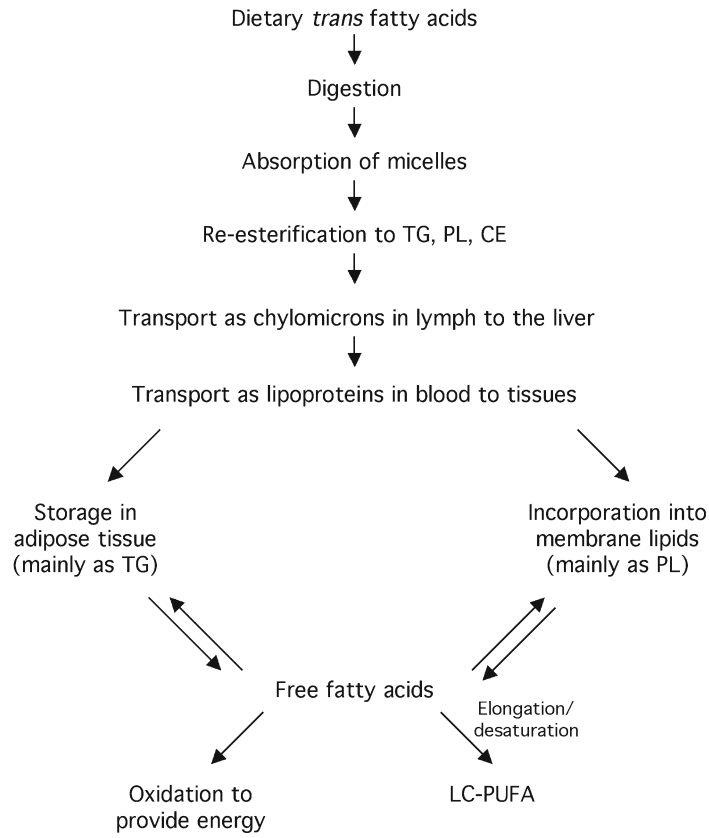

Fig. 3. Simplified scheme of *trans* fatty acid metabolism in humans. TG, triacylglycerols; PL, phospholipids; CE, cholesteryl esters; LC-PUFA, long-chain polyunsaturated fatty acids.

3.2. Tissue Levels

As *trans* fatty acids are not made by humans, tissue levels reflect dietary intakes. The *trans* fatty acid content of adipose tissue for US subjects is about 4% of total fatty acids *(8)*. Approximately 70% of the *trans* fatty acids are C18:1 isomers with double bonds at the Δ–8, –9, –10, –11, –12, or –13 positions. *Trans* C16:1 isomers, which originate mainly from dairy fat, are also found in adipose tissue. About 20% of the *trans* isomers are *trans* C18:2 isomers. Both mono–(Δ–9c, 12t, and Δ–9t, 12c) and di-*trans* (Δ–9t, 12t) C18:2 isomers and several *trans* C18:3 isomers have been detected in adipose tissue. *Trans* C18:1 and C18:2 fatty acids have been found in human kidney, brain, heart, liver, aorta, and jejunum as well ashuman milk *(9)*, and *trans* C18:3 has been detected in platelets and human milk *(10,11)*.

3.3. Conversion

Human liver microsomal complexes contain three different desaturation enzymes: Δ–9, –6, and –5 desaturases, which insert double bonds at the Δ–9, –6, or –5 position of the fatty acid molecule, respectively. Furthermore, fatty acids can be elongated by addition of a two-carbon unit. In this way, stearic acid, linoleic acid, and α-linolenic acid are converted into their longer chain metabolites (Fig. 4), which play an important

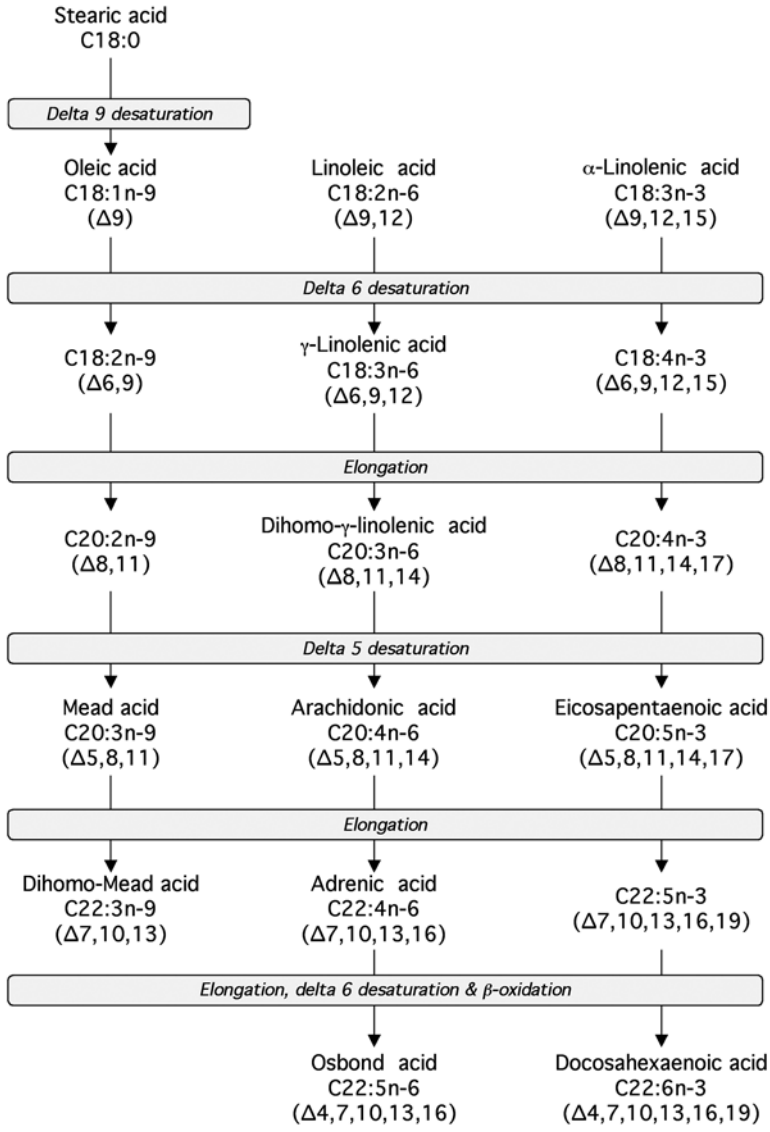

Fig. 4. Desaturation and elongation of fatty acids.

role in many physiological processes. Because chain elongation and desaturation always occur at the carboxyl end of the fatty acid molecule, the position of the first double bond at the methyl end will not change during conversion reactions.

Trans fatty acids are desaturated by the same enzymes as *cis* fatty acids. With the exception of Δ–8, –9, and –10 *trans*-isomers, C18:1 positional isomers are good substrates for Δ–9 desaturase *(12)*. Consequently, *cis, trans* and *trans, cis* fatty acids can be formed. Some *trans*-isomers of C18:1 are also substrates for Δ–6 and –5 desaturases *(13)*. In addition, C18:1 *trans*-isomers can be elongated into C20 and C22 fatty acids.

Trans linoleic acid can be converted into *trans*-isomers of arachidonic acid (C20:4 n-6), although at a lower rate than all-*cis* C18:2 *(14)*. *Trans*-isomers of α-linolenic acid can be converted into *trans*-isomers of docosahexaenoic acid (C22:6 n–3) *(15)*.

Because *trans* and *cis* fatty acids are converted by the same desaturase and elongase enzymes, competition between fatty acids exists. In vitro studies do suggest that both the *cis* and *trans* monoenoic positional isomers inhibit Δ–6 and −5 desaturation of linoleic and α-linolenic acids and their longer chain metabolites. In contrast, C18:1 Δ–9tr increased Δ–9 desaturation of stearic acid, whereas C18:1 Δ-11tr and C18:2 Δ–9tr, 12tr had no effects on Δ–9 desaturase activity *(16,17)*. It is difficult to extrapolate these in vitro findings to the in vivo situation. However, a recent human study found no effect of *trans* α-linolenic acid on the conversion of linoleic acid *(18)*. Therefore, it seems that in adult volunteers consuming a diet adequate in essential fatty acids, the present intake of *trans*-isomers does not affect desaturase activity.

3.4. Oxidation

Only a few human studies have been carried out to examine the postprandial oxidation of *trans* fatty acids. As compared to their *cis* counterparts, *trans*-isomers of oleic and α-linolenic acids were oxidized to the same extent, whereas isomerization increased the postprandial oxidation of linoleic acid *(19,20)*.

4. EFFECTS OF *TRANS* MONOUNSATURATED FATTY ACIDS ON SERUM LIPIDS AND LIPOPROTEINS

4.1. Serum Total, LDL, and HDL Cholesterol

Effects of dietary *trans* monounsaturated fatty acids on serum cholesterol concentrations have been investigated since the early 1960s. Most studies found increased serum total cholesterol levels in humans consuming partially hydrogenated vegetable oils. However, results were not uniform. In addition, these earlier studies did not examine the relationship between dietary *trans* monounsaturated fatty acids and the distribution of cholesterol over the different lipoproteins. In one of the first controlled intervention studies that specifically examined the effects of *trans* monounsaturated fatty acids on serum LDL and HDL cholesterol, 25 men and 34 women were fed a diet that was high in oleic acid, in *trans*-isomers of oleic acid, or in a mixture of the saturated fatty acids lauric and palmitic acid *(21)*. Each diet was administered for 3 wk. The level of *trans* monounsaturated fatty acids in the *trans* diet was 11% of total energy. Results showed that *trans* monounsaturated fatty acids significantly raised serum total and LDL cholesterol levels by 0.26 and 0.37 mmol/L, respectively, *and* lowered HDL cholesterol levels by 0.17 mmol/L compared to oleic acid. These effects were confirmed by later studies that tested lower intakes of *trans* monounsaturated fatty acids. Recently, a meta-analysis was performed that combined the results of all recent studies regarding the effects of *trans* fatty acids on serum lipoproteins. It was estimated that each additional percent of dietary energy as *trans* monounsaturated fatty acids at the expense of oleic acid results in an increase in LDL cholesterol levels of 0.049 mmol/L and a decrease in HDL cholesterol levels of 0.008 mmol/L (Fig. 5) *(22)*.

Consumption of *trans* fatty acids also decreases LDL particle size *(23)*. This is another reason to keep the intake of *trans* fatty acids as low as possible, because small, dense LDL is a risk marker for CHD. Similarly, an elevated fasting triacylglycerol concentration is also a risk marker for CHD. It has been estimated that each additional percent of energy as *trans* monounsaturated fatty acids at the expense of oleic acid increases triacylglycerol levels by 0.019 mmol/L *(22)*.

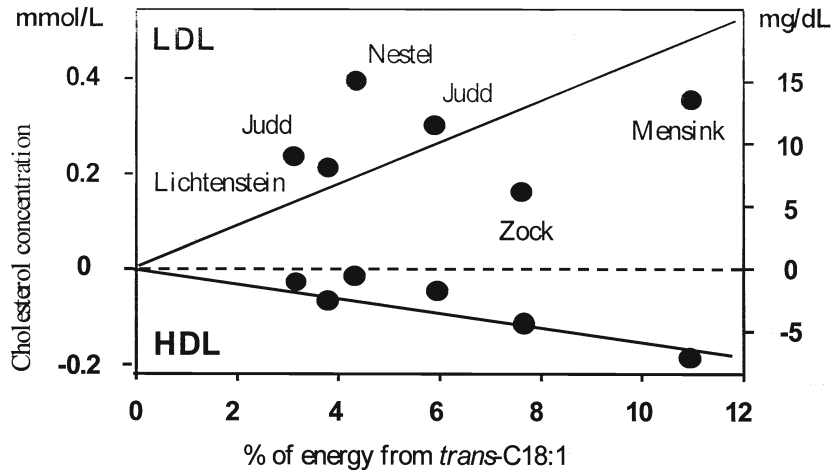

Fig. 5. Effects of exchanging one percent of energy from saturated or *trans* monounsaturated fatty acids for oleic acid on serum total, low-density lipoprotein, and high-density lipoprotein cholesterol and on triacylglycerol concentrations. (Data are derived from ref. *22*.)

Finally, effects of *trans* monounsaturated fatty acids on lipoprotein(a) [Lp(a)] have been studied. Lp(a) is an LDL particle with an extra glycoprotein [apoprotein(a)], which is attached through a disulfide link. High levels of Lp(a) increase the risk for CHD. Therefore, effects of *trans* monounsaturated fatty acids on Lp(a) have been investigated in several intervention studies. From these studies, it can be concluded that when compared with saturated fatty acids, *trans* monounsaturated fatty acids raise Lp(a), particularly in subjects who already have increased Lp(a) levels *(21)*.

4.2. Conclusion

Trans monounsaturated fatty acids do have adverse effects on the serum lipoprotein profile, because they raise serum LDL cholesterol, Lp(a), and triacylglycerol levels, whereas HDL cholesterol levels and LDL particle size decrease. Although they have been studied less extensively, *trans* PUFAs also seem to have unfavorable effects on the serum lipoprotein profile *(24)*.

4.3. Mechanism

The mechanisms underlying the effects of *trans* fatty acids on serum lipoprotein concentrations are poorly understood. It has been hypothesized that cholesteryl ester transfer protein (CETP) is involved. CETP transfers cholesteryl esters from HDL to the apolipoprotein B-containing lipoproteins LDL and VLDL in exchange for triacylglycerol. Indeed, an increased transfer of cholesteryl ester transfer activity has been found in volunteers who consumed a *trans* fatty acid-enriched diet *(25)*.

Lecithin:cholesterol acyltransferase (LCAT) has also been suggested to be involved in this process. LCAT, which is bound to HDL, esterifies free cholesterol from tissues by the transfer of an acyl group from phosphatidylcholine (PC). Because a significant part of the dietary *trans* fatty acids is incorporated into PC *(7)*, a few studies investigated the

effect of *trans* fatty acids on LCAT. One study showed that *trans* fatty acids decreased LCAT activity *(26)*, whereas another study did not demonstrate an effect of *trans* fatty acids on LCAT activity *(27)*.

In the liver, fatty acids from chylomicrons are esterified to free cholesterol by acyl-CoA:cholesterol acyl transferase (ACAT). The affinity of ACAT differs for different fatty acids. A low affinity of the enzyme for a particular fatty acid increases the proportion of free cholesterol in the liver, which decreases LDL receptor activity. Because the LDL receptor removes LDL from plasma, a decreased LDL receptor activity results in higher plasma LDL concentrations. Thus, through this pathway, dietary fatty acids can influence the plasma LDL cholesterol concentration. In hamsters, *trans* monounsaturated fatty acids decreased hepatic cholesteryl ester concentrations relative to oleic acid *(28)*. This suggests that the affinity of ACAT is lower for *trans* monounsaturated fatty acids compared to oleic acid, which may result in higher free cholesterol levels in the liver. In addition, the plasma LDL cholesterol concentration increased and the LDL receptor activity decreased on the diet high in *trans* monounsaturated fatty acids. Therefore, *trans* monounsaturated fatty acids may affect plasma LDL cholesterol via the pathway, as described earlier. However, this mechanism has not been investigated in humans. It is expected that a decreased flux of LDL cholesterol from the blood to cells leads to an increased *de novo* cholesterol synthesis, simply because cells do need cholesterol. However, in healthy volunteers, *de novo* cholesterol synthesis was not different after consumption of a diet high in hydrogenated or unhydrogenated corn oil *(29)*. Thus, the precise mechanism by which *trans* fatty acids influences serum lipoprotein concentrations is unknown.

5. EFFECTS OF *TRANS* FATTY ACIDS ON OTHER RISK FACTORS FOR CHD

5.1. LDL Oxidation

Free radicals initiate the oxidation of unsaturated fatty acids in the LDL particle, which may ultimately lead to formation of an atherosclerotic plaque. Because the fatty acid composition of the LDL particle reflects the fatty acid composition of the diet, effects of increased intakes of *trans* fatty acids on the in vitro susceptibility of LDL to oxidation have been investigated in several intervention trials. However, no effects were found after consumption of *trans* monounsaturated fatty acids compared to other fatty acids *(29)*. Therefore, at least in vitro, *trans* fatty acids have no impact on LDL oxidizability.

5.2. Hemostasis

Only a few human studies have investigated the effects of *trans* fatty acids on markers for platelet aggregation, coagulation, and fibrinolysis—three determinants of hemostatic function. These studies yielded conflicting results, which may result partly from the many different methods to measure hemostatic function. Therefore, it seems that *trans* fatty acids do not have a major impact on hemostatic function *(30–32)*.

6. EPIDEMIOLOGICAL STUDIES

Various approaches have been used in epidemiological studies to examine the relationship between the intake of *trans* fatty acids and the risk for CHD. Because high serum LDL cholesterol levels are positively associated with CHD and high levels of HDL cholesterol are antiatherogenic, some epidemiological studies focused on the relation

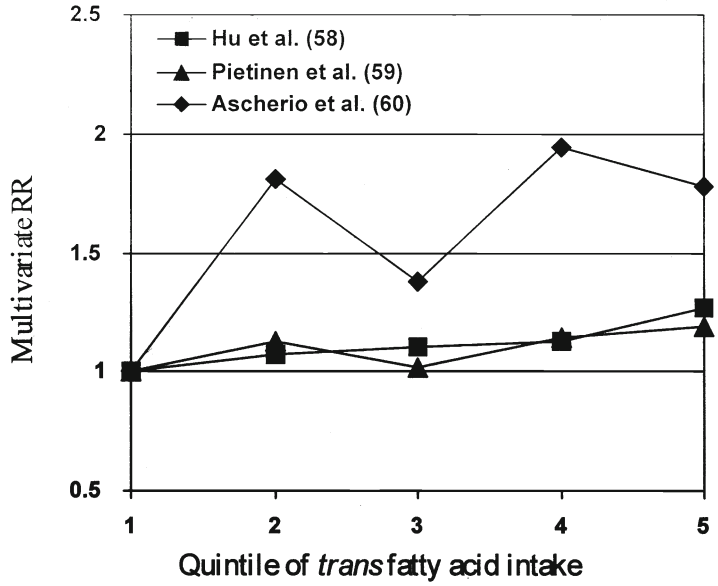

Fig. 6. Pooled relative risk of coronary heart disease, as calculated from the results of four different prospective cohort studies when the intake of *trans* fatty acids is increased with 2% of energy. (Data are derived from ref. *39*.)

between the proportion of *trans* fatty acids in tissues, which reflects dietary intakes, or the dietary *trans* fatty acid intake and the lipid profile *(33–35)*. Altogether, these epidemiological studies confirmed the dietary intervention studies, which found that *trans* fatty acids have a negative impact on the lipid profile. However, these studies did not address the question of whether high intakes of *trans* fatty acids were truly related to the risk for CHD. This issue was examined in several case-control studies. In these studies, the proportion of *trans* fatty acids in tissues or the dietary *trans* fatty acid intake of case-subjects (e.g., subjects with CHD) was compared with that of control subjects (e.g., subjects without CHD). Some studies suggested a higher total *trans* fatty acid intakes *(36)* in cases compared to controls, whereas other studies did not find any effects of *trans* fatty acids on CHD risk *(37)*. One study even showed that the proportion of *trans*-18:1 in adipose tissue was negatively associated with the risk for CHD *(38)*. However, case-controls studies have the disadvantage that they are sensitive to information bias, selection bias, and confounding. Four prospective cohort studies, which suffer less from these disadvantages, examined the relationship between the *trans* fatty acid intake and the risk for CHD. In the Nurses' Health Study *(39)*, the relative risk (RR) of CHD was 1.27 for the highest quintile of *trans* fatty acid intake (mean intake: 2.9% of energy) relative to the lowest quintile (mean intake: 1.3% of energy). This means that women who consume 2.9% of energy as *trans* fatty acids per day have a 27% higher risk for CHD than women with a *trans* fatty acid intake of 1.3% of energy. These results were confirmed by three prospective cohort studies *(40–42)*, shown in Fig. 6. One study looked into details between the source of *trans* fatty acids and the risk for CHD. Relationships for *trans* fatty acids from ruminant and industrial sources were essentially similar *(42)*.

In summary, results from case-control studies are inconsistent. For prospective cohort studies, results are more uniform: a high intake of *trans* fatty acids is associated with an increased risk of CHD.

7. CANCER

Only a few human studies focused on the relationship between *trans* fatty acids and cancer, particularly the risk for breast cancer. In the Nurses' Health Study, a large prospective cohort study, a RR of 0.92 for breast cancer was found when *trans* fatty acid intake increased with 1% of energy *(43)*. Furthermore, three case-control studies related the *trans* fatty acid content of adipose tissue to cancer risk. In the EURAMIC study, the *trans* fatty acid content of subcutaneous adipose tissue was positively associated with risk for breast cancer *(44)*. However, another study suggested a negative association *(45)*, whereas a third case–control study did not find an association between the *trans* fatty acid content of subcutaneous adipose tissue and breast cancer risk *(46)*. Human studies investigating the relationship between *trans* fatty acids and other types of cancers are scarce. In the European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Cancer study, significant correlations were found between *trans* fatty acids in adipose tissue and incidence of colon cancer. No association was found with prostate cancer *(47)*. Therefore, from these human studies, no consistent relationship emerged between the intake and/or tissue levels of *trans* fatty acids and cancer risk.

8. CONCLUSIONS AND RECOMMENDATIONS

The digestion and absorption of *trans* monounsaturated fatty acids are comparable with those of their *cis*-isomers. However, incorporation of various *trans* monoenoic isomers into lipid classes and lipoproteins is lower than that of the *cis*-isomers. Furthermore, both *trans* monounsaturated acids and PUFAs are desaturated and elongated into longer chain metabolites. At present intakes, *trans*-isomers do not affect desaturase activity in adults who consume a diet adequate in essential fatty acids. As compared to their *cis* counterparts, *trans*-isomers of oleic and α-linolenic acids are oxidized to the same extent, whereas isomerization increased the postprandial oxidation of linoleic acid. However, these processes have been investigated mainly in animal and in vitro studies. More human in vivo studies are necessary.

Most studies on the effects of *trans* fatty acids and health have focused on *trans* monounsaturated fatty acids, and effects of *trans* PUFAs are hardly known. *Trans* monounsaturated fatty acids increase serum LDL cholesterol, triacylglycerols, and Lp(a) concentrations and decrease serum HDL cholesterol compared to oleic acid. The exact underlying mechanisms for these effects are not known. Furthermore, *trans* monounsaturated fatty acids seem to have no major impact on LDL oxidizability, platelet aggregation, coagulation, and fibrinolysis. Prospective epidemiological studies showed a positive association between *trans* fatty acids and the risk for CHD but not with the risk for cancer. Based on these results, a reduction in the *trans* fatty acid intake is justified *(48)*.

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IV Diabetes and Obesity

12 Obesity and Insulin Resistance in Childhood and Adolescence

Erik Bergström and Olle Hernell

KEY POINTS

- Obesity in children and adolescents as well as the related metabolic consequences of insulin resistance and non-insulin-dependent diabetes mellitus are increasing worldwide.
- Obesity has become a pediatric problem. Tracking of obesity into adulthood increases with the degree of obesity and age of the child.
- Genetically determined susceptibility makes certain individuals and families more vulnerable than others regarding the development of obesity and its metabolic consequences.
- Besides being a storage depot for fat, adipose tissue is also an endocrine organ with a key function in integrating endocrine, metabolic, and inflammatory signals for the control of energy homeostasis and is mediated through a number of newly discovered bioactive molecules collectively called adipocytokines.
- Increased concentrations of adipose tissue cortisol caused by excess activity of the enzyme 11β-hydroxysteroid dehydrogenase type 1 may contribute to the development of metabolic syndrome in humans.
- Disregarding the molecular mechanisms behind obesity in children, a higher consumption of energy-dense food in combination with a sedentary lifestyle is the major explanation behind the "obesity epidemic."
- Adjusted body mass index is a suitable measure for diagnosis and monitoring of obesity in children and adolescents.
- Early identification, monitoring, and intervention are recommended for children and adolescents with weight problems.
- Programs targeting obesity in children and adolescents should focus on parental guidance skills regarding diet and physical activity in combination with primary preventive efforts to change the "obesogenic" environment.

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1. INTRODUCTION

This chapter focuses particularly on the etiology and pathology of insulin resistance (IR) but also discusses possible preventive measures. Obesity in adults is an increasing health problem in both industrialized and developing countries. In developing countries, obesity coexists with undernutrition, with prevalence rates higher in urban than in rural populations. Women generally have higher rates of obesity than men in both developed and developing countries. In adults, obesity is associated with increased mortality and morbidity in a number of diseases, the most common being cardiovascular diseases (CVDs) and non-insulin-dependent diabetes mellitus (NIDDM) *(1)*.

In parallel to the development in adults, the prevalence of overweight children and adolescents is also increasing worldwide, illustrating that children and adolescents are part of the worldwide epidemic of obesity. Recent reports show that the trend has escalated in recent decades *(2–9)*. In addition, the proportion of overweight children with severe weight problems, or obesity, is becoming greater *(10)*. The obesity "epidemic" began in the industrialized world but is now also spreading to developing countries. In many countries, about 15 to 35% of the children and adolescents are now classified as being overweight and about 5% are classified as obese. Although most infants and young children who are overweight become nonobese adults without treatment, it is well-documented that severe weight problems in childhood and adolescence track into adulthood. Obesity in childhood has adverse psychological and social consequences (e.g., poor self-esteem, peer problems, and social marginalization; refs. *11* and *12*), and a low quality of life that is at the same level as children with cancer *(13)*. It is becoming increasingly clear that childhood obesity also has significant adverse effects on somatic health during childhood *(14,15)*. The risk of becoming an obese adult increases gradually with the age of the child, the degree of obesity, and the presence of a family history of obesity *(2,16)* (Fig. 1).

2. INSULIN RESISTANCE SYNDROME

The clinical entity of obesity, dyslipidemia, and hyperinsulinemia combined with hypertension is often referred to as "IR syndrome" or "metabolic syndrome" (Fig. 2); the syndrome was first described in 1966 by Camus *(17)* and popularized in 1988 by Reaven *(18)*. Although the pathogenesis of this syndrome is multifactorial, genetic factors in combination with a sedentary lifestyle, including a high-energy diet rich in saturated fat and low physical activity leading to obesity, are regarded as major determinants *(17,18)*.

Obesity is related to increased cellular resistance to insulin, which in turn affects glucose and lipid metabolism, resulting in a secondary hyperglycemia, a compensatory hyperinsulinemia and dyslipidemia (i.e., hypertriglyceridemia), and low high-density lipoprotein lipoprotein (HDL) concentration. Insulin-stimulated glucose uptake and utilization is impaired in liver, skeletal muscle, and adipose tissue, and coupled to impaired suppression of hepatic glucose output. IR also affects the regulation of blood pressure and the fibrinolysis system *(19–21)*. The exact molecular mechanisms behind IR remain unclear, but there seems to be a genetic component *(22)*. Changes in plasma free fatty acid (FFA) concentration may be an important link between obesity and IR *(23–26)*. The metabolic disturbances caused by IR are related to increased risk of CVD and NIDDM. One important mechanism could be that IR leads to inadequate intracellular

Fig. 1. Odds ratio for obesity in young adulthood according to the child's age and obesity status of the child. Nonobese children served as reference group. (From ref. *16*.)

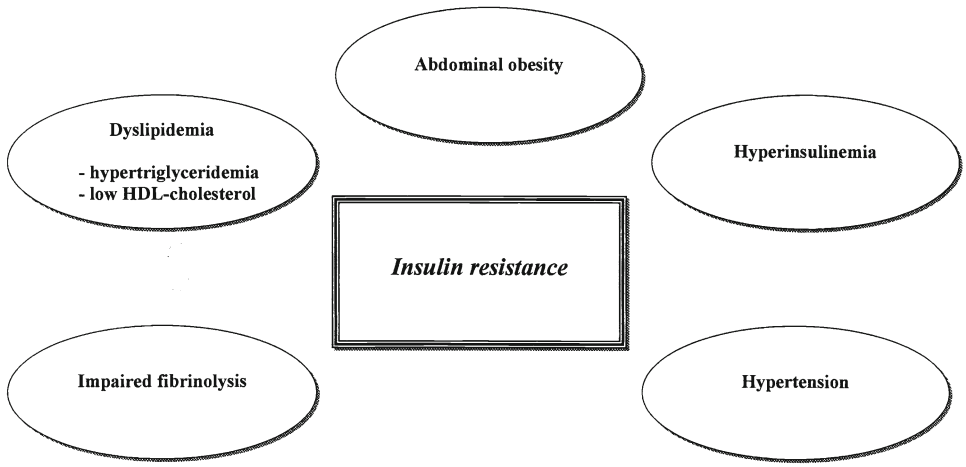

Fig. 2. The insulin resistance syndrome.

glucose concentration, which in turn leads to insufficient amounts of adenosine triphosphate (ATP) that is needed for ion transfer and energy-requiring reactions *(27)*. The degree of the weight problem, the change in weight, and the duration of the weight problem are all separate predictors of NIDDM. The metabolic syndrome seems to be related more to obesity than to heredity of IR, but it should also be noted that IR is related to CVD regardless of obesity *(23,28,29)*. The metabolic disturbances seen in the metabolic syndrome are especially related to abdominal obesity or, more precisely, increased visceral fat, even in normal-weight individuals. Genetic and/or familial

nongenetic factors seem to influence both visceral fat mass and plasma insulin levels to a certain degree *(30–32)*. Magnetic resonance imaging appears to be a convenient technique to estimate visceral fat mass *(33,34)*.

There is increasing evidence supporting the view that the metabolic aberrations seen in the adult metabolic syndrome can be identified early in childhood and tracked into young adulthood *(35–42)*. Heredity, as well as ethnicity, seems to have an impact, with a stronger effect of obesity on IR in African-American rather than Caucasian adolescents *(43)*. However, it is not yet known at what levels serum insulin, serum lipids, blood pressure, or weight problems begin to increase the risk of later adult disease. Indices of obesity (body mass index [BMI], skinfold, waist circumference), independent of age, correlate positively to serum insulin and an atherogenic serum lipid profile (i.e., elevated total cholesterol, low-density lipoprotein cholesterol [LDL-C], serum triglyceride, and apolipoprotein B [apo-B]) and subnormal HDL cholesterol (HDL-C). Waist-to-hip circumference or subscapular-to-triceps skinfold ratios seem to have no advantage to BMI or waist circumference alone as an indicator of unfavorable metabolic or physiological values. BMI and waist circumference are also easy measures to obtain, making them particularly suitable for epidemiological studies *(35,44)*. The importance of combining BMI with waist measurements is emphasized by the strong relationship between visceral fat and IR, which is also demonstrated in adolescents *(34)*. Using only BMI may cause underestimation of central obesity *(45)*.

There are no available reference values for serum insulin in healthy children and adolescents. Serum insulin levels increase during puberty, probably as a result of a combination of higher secretion, a higher demand of insulin during the pubertal growth spurt, and increased cellular resistance to insulin *(46)*. However, the physiological explanation for increased cellular resistance to insulin in puberty is not known. Still, insulin is known to have a growth hormone-like effect, and increased levels may result from increased secretion of insulin related to the pubertal growth spurt. At any given age, girls have higher values compared to boys, probably as a result of differences in maturation *(35)*. There is also an intraindividual diurnal variation in serum insulin, partly caused by variation in daily dietary intake. These variations make comparisons between different studies difficult. Insulin-like growth factor binding protein-1 level is strongly associated with insulin sensitivity and may be a useful early predictor of development of IR in prepubertal obese children *(47)*.

Studies using insulin clamping technique and glucose tolerance tests have shown obese adolescents to have major deficits in insulin-stimulated oxidative and nonoxidative glucose metabolism, hyperinsulinemia (both in the fasting state and in response to intravenous glucose), and impaired suppression of total body lipid oxidation and plasma FFA concentrations in response to insulin *(34,38)* (Fig. 3). An extension of these metabolic changes is NIDDM, and, accompanying the national rise in adolescent obesity, there are reports of increasing incidence of NIDDM among adolescents *(48,49)*. It seems that the disease develops faster in children than in adults *(42)*.

3. ASSESSMENT OF OBESITY

In adults, the BMI (kg/m^2) has become the standard index for assessing obesity *(1)*. Several suggestions have been made on how to assess obesity in children, such as standard deviation (SDs) scores of weight, weight-to-height ratios or indices, and skinfold measurements and ratios. All of these anthropometric measurements are

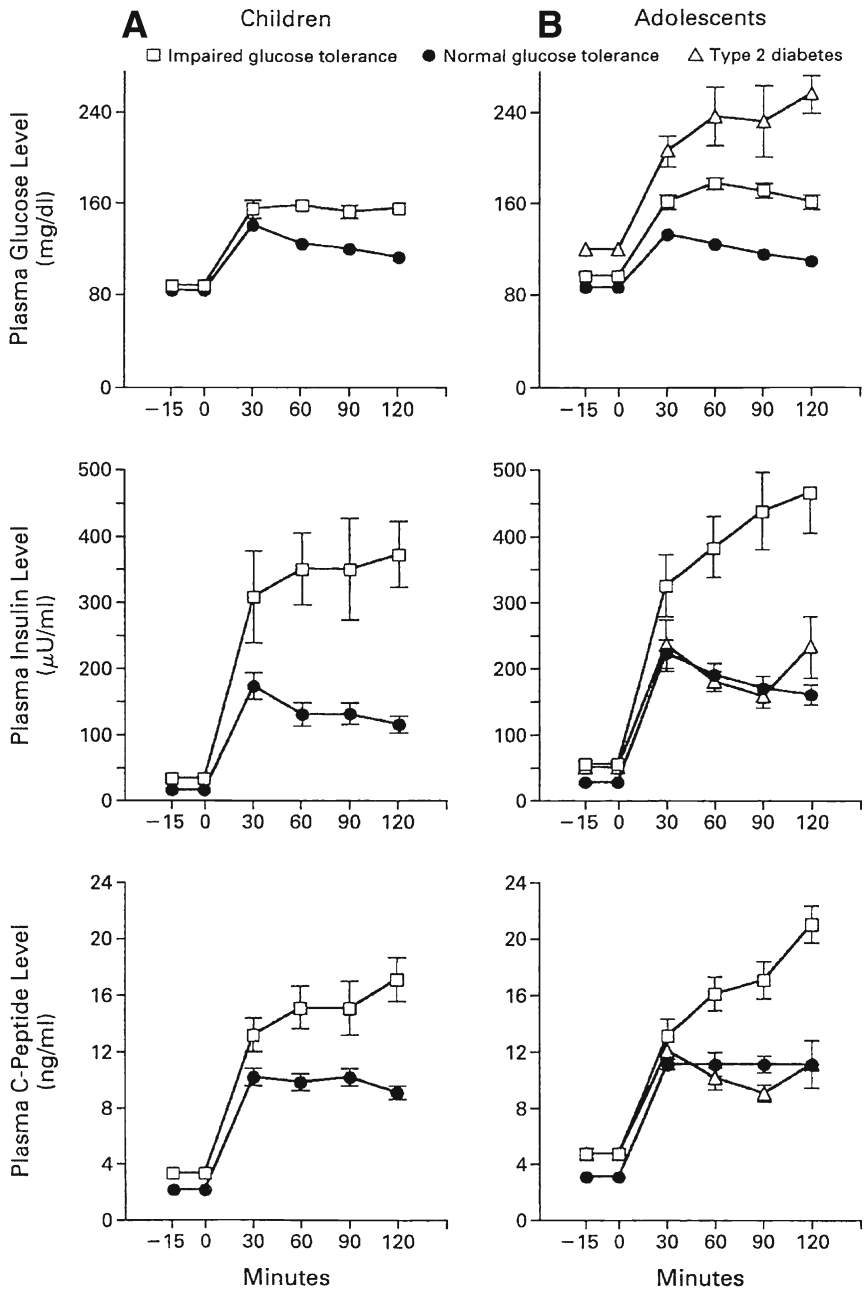

Fig. 3. Mean plasma glucose, insulin, and C-peptide responses during oral glucose-tolerance test in obese children **(A)** and adolescents **(B)** with normal glucose tolerance, impaired glucose tolerance, or type 2 diabetes mellitus. (From ref. *38*.)

dependent on gender, age, and physical development of the individual. Because weight is closely related to height, when evaluating obesity, it is always necessary to consider height and weight together. Of course, in severe cases, obesity is obvious simply by inspection.

The height and weight growth chart is the most commonly used instrument in the evaluation of obesity in children and adolescents *(50)*. By comparing the current weight of the child with the reference weight in the chart according to the height of the child, a judgment that the child is overweight may be made. One limitation has been the lack of distinct cut-off values. In the last years, however, BMI also has become used increasingly in children and adolescents. Because there is a normal variation in BMI with age (a J-shaped curve with a nadir around age 5 yr and then increasing continuously up to adult values), BMI values in children and adolescents need to be assessed using age-related reference curves. Such curves have been constructed in different countries *(51–53)*. It should be noted that national BMI curves are relevant only for the reference population from which the data was extracted. Therefore, the 85th and 95th percentile values differ depending on ethnicity and the nutritional status of the population *(54)*. When using BMI in developing countries, it is important to consider the effect of stunting, as the lower height of the child results in a relatively higher BMI without presence of obesity. The 85th and 95th percentiles in BMI have commonly been used to classify weight problems and obesity, respectively *(55)*. It has been suggested that early BMI rebound (age <5 yr) is a useful predictor because it relates to adult obesity *(56)*. However, this has been questioned as an "epiphenomenon" because children with higher BMIs do have earlier rebound than those with lower BMIs, indicating that it is not an early rebound *per se* but rather a high BMI that increases the risk of later obesity *(57)*. Recently, a new international standard was introduced called the adjusted BMI (iso-BMI), which is based on pooled international data for BMI of children from six countries *(58)*. From these data, percentile curves were derived linking childhood BMI values for each age and gender to BMIs of 25 and 30 kg/m^2 at age 18 yr (iso-25 and iso-30; i.e., the commonly used cut-off values for overweight and obese adults, respectively). This new standard is now used for classification of overweight and obese children in international comparative studies *(8)*.

4. ETIOLOGY OF OBESITY

Obesity is the result of energy imbalance; stored energy equals the difference between energy intake and energy expenditure (that is, resting metabolic rate and physical activity). Although it is evident that obese individuals eat more than they require, there is increasing evidence that there are genetically determined metabolic differences between individuals who gain extensive weight and those who do not *(59–63)*. However, it is obvious that the increase in obesity prevalence and severity in different populations seen during recent decades cannot be explained by genetic changes; rather, the increases are caused by environmental and societal changes affecting energy intake and expenditure.

4.1. Regulation of Body Weight

Body weight is regulated by complex interactions of endocrine and neural signals from adipose tissue and the endocrine, neural, and gastrointestinal systems (Fig. 4). Afferent neural (vagal and cholinergic) and hormonal (insulin, cholecystokinin, leptin, glucocorticoids [GCs]) stimuli related to metabolic status are received in the hypothalamus, where they modulate the release of peptides known to affect food intake and efferent signals to the hypothalamic–pituitary axis, resulting in energy expenditure and insulin release *(61)*. In the normal state, body weight is regulated so that energy expenditure

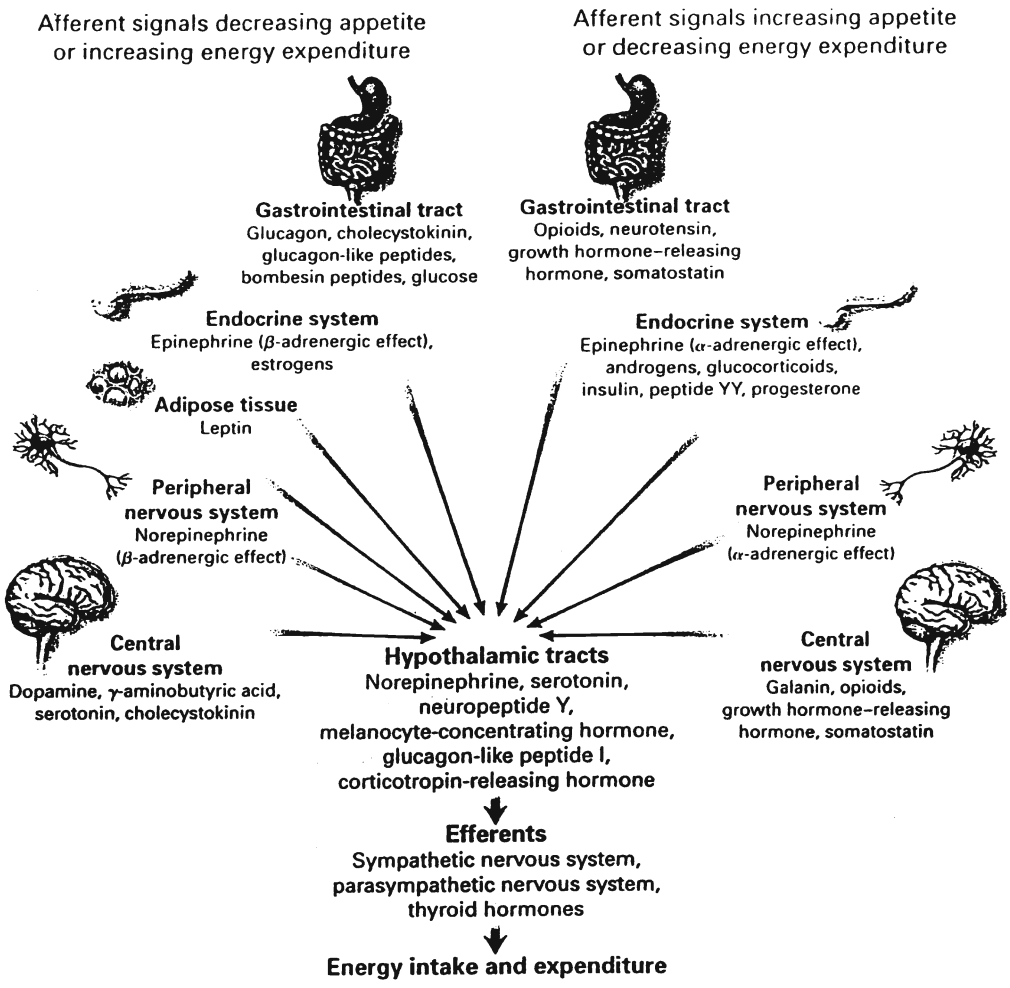

Fig. 4. Molecules that affect energy intake and expenditure. (From ref. *61*.)

equals energy intake. If energy intake exceeds expenditure for a long time period, the surplus of energy is stored as fat and, if the energy imbalance continues, the result is obesity. Once the obese state is established, the new weight appears to be defended, resulting in an involuntary, physiologically driven increase in energy intake and difficulties in losing weight *(64)*.

4.2. Genetics

Regulation of body weight is under strong genetic influence. The obesity gene map shows putative loci on all chromosomes except the Y chromosome *(65)*. Some rare disorders of energy balance have been identified that arise from genetic defects. The discovery of these genetic defects has biological and clinical implications beyond the importance they deserve, because although they are rare diseases, they have the potential to contribute general knowledge and increase our understanding of mechanisms regulating energy intake and expenditure *(66)*.

Obesity, diabetes, and CVD aggregate in some families; however, family members do not share only the same genes but also, to various extents, the same environment and habits. However, studies on twins, adoptees, and families also show a strong correlation between body weights of relatives when they have not shared the same environment. As much as 70 to 80% of the variance in BMIs may be attributable to genetic factors. The heritage of obesity is mediated through factors such as resting metabolic rate, changes in energy expenditure in response to overeating, lipoprotein lipase activity, maximal insulin-stimulated glyceride synthesis, basal rates of lipolysis, adipose tissue distribution, certain aspects of eating behavior, food preferences, and level of physical activity *(61)*. Although there are examples of cases of obesity in rodents resulting from single-gene mutations in metabolic pathways, obesity in humans is rarely attributable to a single gene (e.g., Prader-Willi Syndrome). According to Loos and Boucard *(63)*, human studies to identify the specific genes involved in common obesity currently are dominated by three types of strategies: (a) the candidate gene approach, which relies on the current understanding of the pathophysiology of obesity related to energy balance and/or to adipose tissue biology; (b) genome-wide linkage scans with a view to identifying chromosomal regions of interest, the so-called quantitative trait locus (QTLs), and eventually genes within these QTLs; and (c) tissue-specific gene expression profile comparing lean vs obese individuals and other informative samples *(63)*. Because disturbance of energy balance is the central pathology of obesity, the search for candidate genes has focused on those that are involved in energy metabolism, (i.e., genes that program the β_3-adrenergic receptor, the GC receptor, and Na+/K+ ATPase), obesity syndromes in humans, obesity genes in rodents (e.g., genes coding for leptin and the leptin receptor) and genes involved in regulation of response to an obesity-promoting environment *(61,64,67)*. Generally, obesity must be considered as a complex polygenic disease resulting in different genetic predisposition, or susceptibility, for obesity. In a low "obesogenic" environment, i.e., an environment favoring physical activity and a balanced energy intake, only individuals with a high predisposition will become obese, whereas in an environment favoring sedentary behavior and excess energy intake, individuals with a low predisposition also will become obese. Only a small fraction of the population seems to be genetically "protected" from becoming obese *(63,68)*.

4.3. Adipocytokines

Rather than being merely an inert storage depot for fat, adipose tissue is now being recognized as an important endocrine organ with a key role in the integration of endocrine, metabolic, and inflammatory signaling for the control of metabolic homeostasis. Fat cells synthesize and secrete into the circulation a variety of "bioactive proteins," some of which have been identified only recently and are referred to collectively as "adipocytokines," e.g., leptin, tumor necrosis factor-α, plasminogen activator-inhibitor type I, adipsin, resistin, and adiponectin *(69)*. The function of these and their role in obesity development and IR is currently the subject of intense research and is beyond the scope of this chapter. However, leptin is discussed here as an example of their potential importance.

Leptin was one of the first adipocytokines to be identified and is regarded as a critical link between somatic energy stores and growth and fertility. Leptin is the adipocyte-specific

product of the *ob* gene (*Lep* gene), first identified in the leptin-deficient, obese *ob/ob* mouse *(70)*. It is thought to mediate afferent signals from fat stores to the brain, resulting in subsequent efferent signals that regulate food intake and energy expenditure. Mutations in the leptin or leptin receptor genes result in morbid obesity, infertility, and IR in rodents and humans, but most obese humans do not have abnormal leptin or leptin receptor concentrations or functions. However, plasma leptin concentrations are increased in obese humans in direct proportion to body fat mass, and leptin is believed to influence whole body glucose homeostasis and insulin action and may be involved in the pathogenesis of the metabolic syndrome *(71–73)*.

Serum leptin levels are higher in obese than in lean children, higher in girls than in boys, and has been related to IR *(74)*. Obese children, especially girls, tend to mature earlier than lean children, and obese children also have increased adrenal androgen levels, which may partly explain the accelerated growth of these children before puberty. Recent data indicate that leptin has a specific role in stimulating the activity of enzymes that are essential for the synthesis of adrenal androgens *(75)*.

4.4. Glucocorticoids

As mentioned earlier, the adverse metabolic consequences of obesity are best predicted by the quantity of visceral fat. The clinical resemblance between the metabolic syndrome and Cushing's syndrome has focused much interest on the potential causative role of GCs in obesity and IR. However, in typical obesity, plasma GC levels are not elevated. Recently, the role of GCs gained renewed interest from the observation that transgenic mice overexpressing the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in adipose tissue (which converts inactive circulating 11-keto steroids into active glucocorticoids, e.g., cortisone to cortisol) have increased intracellular cortisol levels and develop central obesity, IR, hypertension, hyperglycemia, hyperlipidemia, and hyperphagia despite having elevated circulating leptin levels *(22)*. Conversely, 11βHSD1 knockout mice have low intracellular GC levels and are protected from obesity, diabetes, and dyslipidemia *(76)*. Indeed, in adipose tissue of obese humans and rodents, 11β-HSD1 is elevated, indicating that adipose tissue GC excess resulting from dysregulation of 11βHSD1 may contribute to the development of visceral obesity and the metabolic syndrome in humans *(77–79)*. We recently found higher expression levels of 11βHSD1 in visceral as compared to subcutaneous adipose tissue in overweight children, suggesting that such an effect also might be relevant in childhood obesity (Li X, Lindquist S, Chen R, Olsson T, Hernell O, unpublished).

4.5. Thrifty Genotype and Thrifty Phenotype

Although there seems to be a stronger genetic influence in the etiology of obesity than previously believed, epidemiological data showing large differences in obesity prevalence and trends between countries demonstrate the additional contribution of environmental factors. This is further supported by variation in rates by ethnicity and socioeconomic status and the shift in rates seen in migrant populations *(1)*. One hypothesis that might explain the epidemics of lifestyle-related diseases (e.g., CVD and NIDDM) is the thrifty genotype. As stated by Neel, "Genes and combinations of genes which were at some time an asset may in the face of environmental change become a liability" *(80)*. An energy-saving genotype and the preference for energy-dense food

(food rich in fat and sugar) is a survival and reproductive advantage given an environment with a shortage of food but not when confronted with the surplus of Western lifestyle and society *(81)*. This hypothesis is supported by the absence of CVD and obesity in native populations still unaffected by Western lifestyle *(82)* and by the fact that the highest obesity and NIDDM prevalence is found in populations that have undergone rapid alterations in lifestyle (e.g., Australian Aborigines, Native Americans, Pacific Islanders, and some migrant populations such as Asian Indians) *(1)*.

There is no doubt that besides genetics, the chief cause of obesity, NIDDM, and CVD is a sedentary lifestyle and excess food intake in adult life. However, increasing evidence suggests that nutritional circumstances (both nutritional deficiency and excess) early in life also matter , and that the effect seems to be different depending on in which phase in fetal life the nutritional disturbance occurs. This hypothesis has been named the thrifty phenotype hypothesis *(83)*. In a historical cohort study of young men exposed to the Dutch famine of 1944–1945 and examined at military induction, Ravelli showed that during the last trimester of pregnancy and the first months of life, exposure to famine produced significantly lower obesity rates, whereas during the first half of pregnancy, exposure to famine resulted in significantly higher obesity rates *(84)*. A similar observation from the 1970s was made by Forsdahl *(85)*, who demonstrated regional associations between infant mortality and CVD mortality in adults in the same regions a generation later. Later retrospective and ecological studies *(86–89)* confirmed the associations between reduced physical growth in early life and CVD mortality in adulthood, extending this also to associations between poor fetal and infant growth and elevated serum lipids, higher blood pressure, impaired glucose tolerance, higher fibrinogen levels, higher incidence of NIDDM, and the metabolic syndrome. Other reports showed that low birth weight in conjunction with rapid postnatal weight gain , especially as subcutaneous fat, is associated with reduced glucose tolerance in childhood and, consequently, susceptibility for NIDDM later in life *(90–93)*. Furthermore, stunted children seem to have a higher risk of obesity *(94)*.

Referring to animal experiments, Barker *(87)* proposed a biological explanation for the association between low birth weight and infant growth and later metabolic diseases. Barker suggested that fetal malnutrition results in a permanent underdevelopment of certain organs (e.g., the liver and pancreas) or, alternatively, an early metabolic or nutritional "programming." Both could result in difficulties in coping with a nutritional overload later in life. If fetal malnutrition results in increased risk of CVD, the low occurrence of adult CVD in developing countries with concurrent malnutrition and high rates of intrauterine and infant growth retardation may seem paradoxical. However, this could be explained partly by a shorter life span in developing countries (too short for chronic diseases to appear) and by adult populations in these countries not having been exposed to nutritional overload. According to Barker *(87)*, transition from nutritional insufficiency to nutritional overload may be particularly harmful. This mechanism could be an additional explanation for the rapid increase in obesity and CVD in native populations.

The molecular mechanisms underlying the suggested links between fetal and infant malnutrition and later metabolic disorders are unknown, but it has been demonstrated that supraphysiological doses of GCs retard fetal growth and that human intrauterine growth retardation is associated with elevated cortisol levels. Exposing rats to excessive GCs *in utero* reduces birth weight and causes permanent hypertension in the

adult offspring. Therefore, GCs could be one link between low birth weight and later disease *(95)*.

The programming, or imprinting, hypothesis has been disputed; the main criticism is the lack of proper control for confounding by later dietary and other socially determined environmental factors *(96)*. Even if the programming hypothesis suggests that experiences *in utero* or in early life may permanently affect the risk of developing CVD in adulthood, this hypothesis does not exclude the risk of also being affected by environmental factors or lifestyle later in life. One factor that was recently discussed was duration of breastfeeding, where an increasing number of studies show that breastfeeding can have long-term benefits, including prevention of obesity, although clear evidence is still lacking *(97,98)*.

In contrast to these reports and also indicated in the Dutch Famine study are reports that show a positive association between birth weight and BMI in adulthood *(99–101)*. For example, adolescents with high serum insulin levels were heavier and taller at birth and during infancy and childhood compared to those with lower serum insulin values *(35)* (Fig. 5). A possible explanation for this finding is that high insulin values may stimulate growth. Alternatively, tall and heavy children may have higher insulin values. A corresponding finding, which also illustrates the positive association between physical growth and glucose metabolism, showed higher linear growth prior to the onset of insulin-dependent diabetes in children *(102)*. Another possible explanation that should be considered is that a high BMI or weight may also indicate a high lean body mass *(103)*. Furthermore, maternal weight (or BMI) largely explains the association between birth weight and the child's adult BMI, and it may be a more important risk factor for obesity in the child than the birth weight of the child *(100,104)*. Intergenerational associations between the mother's and offspring's BMIs may be an important association fueling the obesity epidemic, that is, an increasing number of mothers having an increasing number of girls who become an increasing number of obese mothers, and so on.

4.6. Diet

The increase in obesity in various populations is associated with a change to a Western diet. Compared to traditional diets of native populations, a Western diet contains increased amounts of total fat—particularly animal fat—animal protein, salt, and sugar, and decreased amounts of vegetable fat, vegetable protein, and complex carbohydrates. This implies a change in both composition and energy density of the diet *(105)*.

5. ENERGY INTAKE

It is indisputable that a high-energy diet that exceeds the energy demands results in obesity, especially in combination with a low level of physical activity. However, most dietary studies show no correlation (or one that is inverse) between reported energy intake and body weight *(60)*. The common explanation for this has been a higher degree of underreporting of dietary intake in obese individuals. Studies in children indicate that obese children do report a relatively lower energy intake than lean children. Obese adolescents underreported their dietary intake more than adolescents of normal weight when their 7-d self-reported records were compared with estimated energy expenditure using the double-labeled water method *(106,107)*. Comparing three dietary surveys in Sweden over the past 20 yr, mean energy and fat intakes seemed to decrease, a trend

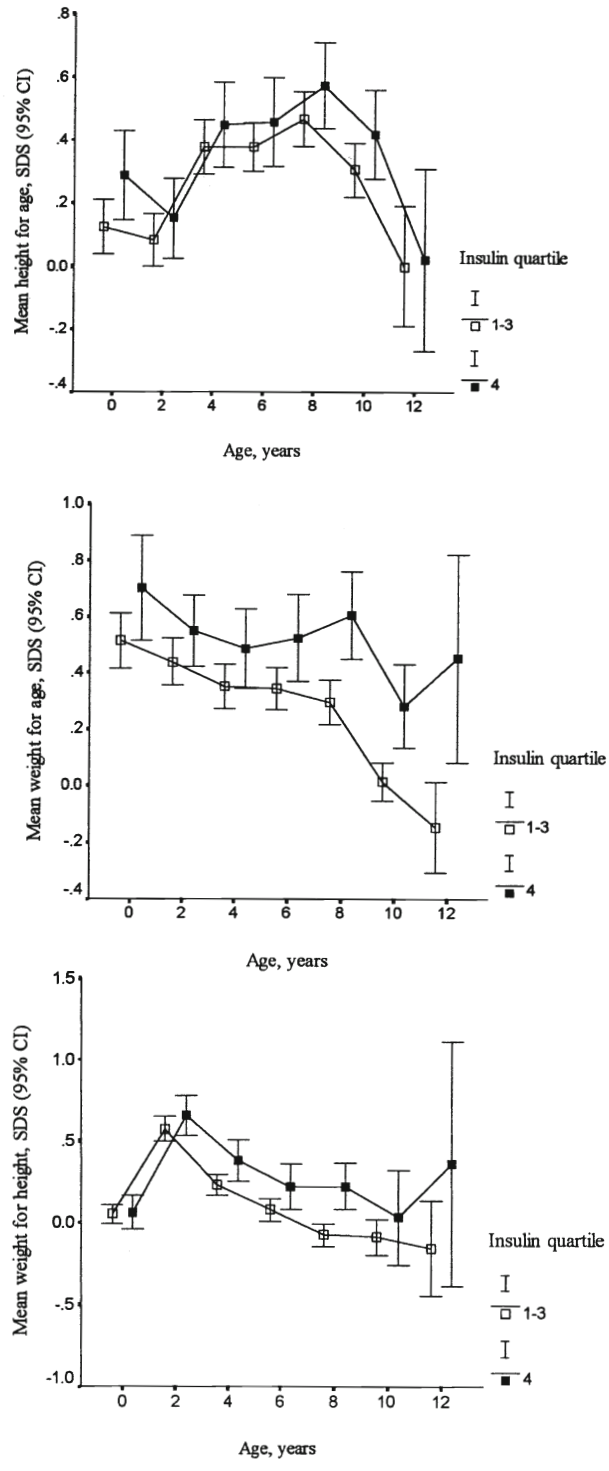

Fig. 5. Relationship between s-insulin levels in adolescence and previous attained heights and weights during infancy and childhood. (From ref. *35*.)

also reported from other countries *(108–111)*. Thus, another likely explanation for increased obesity prevalence could be a gradual reduction in physical activity over the past few decades. However, even if the relatively low energy intakes found in obese individuals is partly an effect of underreporting, it may also be a reflection of metabolic differences between lean and obese individuals. In fact, there may not be such large difficulties in energy intake between obese and lean individuals.

6. METABOLIC EFFECTS OF COMPOSITION OF DIET

There are some characteristics of macronutrients that may explain part of the difficulties of regulating food intake when food is available in excess. Humans seem to have a poor defense against excess intake of fat. Compared to carbohydrates, fat has a lower ability to suppress hunger and bring eating to an end, and there are limited metabolic pathways that transfer excess intake of fat to other compartments, including a low ability to stimulate increased oxidation on intake. At the same time, fat is energy-dense and is easily stored at low energy cost *(112)*. A more fat-dense diet has been related to obesity in children *(113)*. Because of rapid growth, infants need a higher energy intake per kilogram of body weight than do children and adults. This is reflected by the high energy density of human milk, in which fat accounts for about 50% of total energy. However, when the growth rate decreases after infancy, food with too high energy density may result in energy overload. There is also some evidence to support the contention that the high intake of protein in the Western diet could, through a specific effect on hormonal status, contribute to the metabolic disturbances behind obesity. Together with a genetic susceptibility for weight gain, this specific effect of high protein intake could partly explain why obese individuals tend to have energy intakes lower than would be expected from their degree of obesity, even when the underreporting of energy intake is accounted for *(114)*. In rats, there is a more persistent increased secretion of glucagon after protein intake compared to the transient increase of insulin and increased glycogenolysis *(115)*. However, although the composition of the food may have some specific effects on development of obesity, the most significant contributing factor appears to be the high energy density of the diet.

6.1. Glycemic Index

There are some reports showing that IR and hyperinsulinemia may occur prior to other manifestations of the metabolic syndrome, indicating that high-fat and refined-sugar diets may have a more direct effect. Monosaccharides, disaccharides, and starch are defined as digestible carbohydrates, because they are digested and absorbed in the human small intestine. A second category of carbohydrates, defined as "nondigestible" (e.g., dietary fiber), cannot be digested by intestinal enzymes. This latter category of carbohydrates, including a fraction of starch called "resistant starch," still may have an important nutritional role because of its inhibitory effect on food intake and, possibly, on weight management. Among the various dietary constituents, the one with the strongest influence on blood glucose levels in the postprandial period is the amount of digestible carbohydrates in the diet. The glycemic index (GI) is a system to classify carbohydrates according to their influence on blood glucose and is defined as the incremental blood glucose area under the curve after a test product has been ingested, expressed as the percentage of the corresponding area after a carbohydrate-equivalent amount of white bread *(116,117)*. Compared with a low-GI meal, a high-GI meal results in higher serum insulin

levels, lower plasma glucagon levels, lower postabsorptive plasma glucose and serum fatty acids levels, and elevation in plasma epinephrine. The rapid absorption of glucose after consumption of high-GI meals induces hormonal and metabolic changes that promote excessive voluntary food intake in obese subjects *(118),* (i.e., a rapid increase in blood glucose results in a hyperinsulinemic response with rapidly falling blood glucose causing increased hunger and overeating) *(119)*. Additional studies are needed to examine the relationship between dietary GI and long-term body weight regulation.

High consumption of carbohydrate-rich foods with high-GIs and sugar may be one cause of the increase in obesity, as the consumption of added sugar, sweeteners, and soft drinks has increased dramatically during the same time period as the obesity epidemic has evolved. However, the evidence is rather weak because data are essentially based on epidemiological observations *(120–122)*. The link seems to be that soft drinks are an easily consumed source of energy. Children who are high consumers of soft drinks have higher total energy intakes than children who are nonconsumers *(123)*, and obese children consume a larger proportion of their total energy intake from soft drinks in comparison with lean children *(110,124)*.

Dietary constituents may delay the process of digestion and/or absorption of carbohydrates in the intestine, thus reducing the GI. A diet that is rich in dietary fiber is recommended to prevent or treat obesity because of its lower energy density, its ability to induce satiety, and its ability to slow the rate of food ingestion, gastric emptying, and digestion, thereby lowering insulin levels and also resulting in a more favorable plasma lipid profile *(125,126)*. Although well-documented in adults, there are few useful studies of these effects of fibers in children. Switching to a diet that is rich in complex carbohydrates and dietary fiber increases peripheral insulin sensitivity *(127–130)*.

6.2. Fatty Acids

Dietary fatty acids have several specific metabolic effects (insulin secretion and action, hemostatic factors and platelet function, blood pressure, and endothelial activation) related to chronic diseases *(130)*. Lowering fat in the diet to 25% of total energy reduces platelet aggregation and significantly increases fibrinolytic activity *(131)*. Elevations of plasma FFA levels produce peripheral, and probably also hepatic, IR in obese subjects who are healthy as well as those who are diabetic. Elevations also increase glucose-stimulated insulin secretion, and this effect also seems to be dependent on chain length and degree of saturation. The longer and more saturated the fatty acids, the more pronounced the insulin response *(26,132)*. A high proportion of palmitic (16:0) and a low proportion of linoleic (18:2 n-6) acids are associated with the metabolic syndrome, indicating that there may be a causal relationship between the type of fat in the diet and insulin action *(133)*.

Although there is substantial agreement that diet (predominantly the amount and type of fat intake) affects serum lipoproteins and, ultimately, the occurrence of CVD, it has been difficult to demonstrate a direct association between dietary fat intake and serum lipid levels *(130)*. An intervention study by Vessby et al. *(134)* demonstrated that insulin sensitivity was significantly impaired on a saturated fatty acid diet but not on a monounsaturated fatty acid diet. The addition of n-3 fatty acids influenced neither insulin sensitivity nor insulin secretion. The favorable effects on insulin sensitivity from substituting a monounsaturated fatty acid diet for a saturated fatty acid diet were only seen at a total fat intake below median. LDL-C increased on the saturated fatty acid diet

but decreased on the monounsaturated fatty acid diet. A change of the proportions of dietary fatty acids (decreasing saturated fatty acid and increasing monounsaturated fatty acid) improved insulin sensitivity but had no effect on insulin secretion, and there seemed to be no beneficial impact of the fat quality on insulin sensitivity in individuals with a high fat intake *(134)*.

In infants and younger children, the association between dietary fat intake and serum lipids seemed to be stronger than in older children *(135,136)*. However, ecological studies comparing different countries showed associations between fat intake in the population and the level of total cholesterol and LDL-C in adolescents. In experimental studies, a reduction of fat intake, or a modification of fat composition regarding atherogenic fatty acids, was shown to lower serum lipids in children *(137,138)*.

There are several possible explanations for the difficulties in showing an association between individual fat intakes and serum lipid levels (e.g., poor dietary data, genetically determined differences in response to fat intake). There may also be a threshold effect, as the effect of fat intake on serum lipids is more pronounced in the low range of fat intake and, therefore, is difficult to show when the population level of fat intake is comparatively high and homogenous *(139,140)*. It should also be considered that atherosclerosis develops over decades and the variation in food consumed would be greater if the exposure time and, hence, the cumulative exposure of different diets was considered. Alternatively, there may be other dietary factors (e.g., antioxidants) that may prove more important, or at least equally important, than fat intake for protection against CVD *(141)*.

6.3. Physical Activity

The major part of daily energy expenditure is explained by basic metabolic energy expenditure, or basic metabolic rate, which constitutes 50 to 70% of daily energy expenditure. Voluntary physical activity accounts for less than 25%, implying that the direct impact of voluntary physical activity on energy expenditure is rather low. The increase in obesity in society parallels the increase in sedentary lifestyles; in children, sedentary activities, especially watching television, are related to obesity *(142)*, indicating a causal relationship. Although there are reports indicating that high physical activity during childhood may reduce gain in body fat in early adolescence *(143),* the evidence is still inconclusive regarding the link between physical activity and obesity *(144)*. For example, the physical activity level of children and adolescents is associated to a number of social and family factors *(145)*.

Physical exercise, even of short duration and especially when combined with a high-complex carbohydrate diet, increases insulin sensitivity, decreases serum insulin and triglyceride levels, and increases HDL-C (Fig. 6). Increased participation in nonvigorous as well as overall and vigorous physical activity significantly reduces the risk of developing NIDDM *(146,147)*. Physical activity is believed to influence the fatty acid composition of phospholipids in skeletal muscle, affecting skeletal muscle membrane fluidity and peripheral insulin sensitivity. Skeletal muscle glucose uptake and metabolism are major determinants of whole-body glucose metabolism in response to exercise and insulin stimulation *(148–151)*.

In epidemiological studies in adults, high levels of physical activity and physical fitness, which usually are not discussed as separate entities, have been associated with lower prevalence of CVD risk factors and a lower incidence of coronary heart disease *(152)*. The positive effect of physical activity on the CVD process is not fully understood;

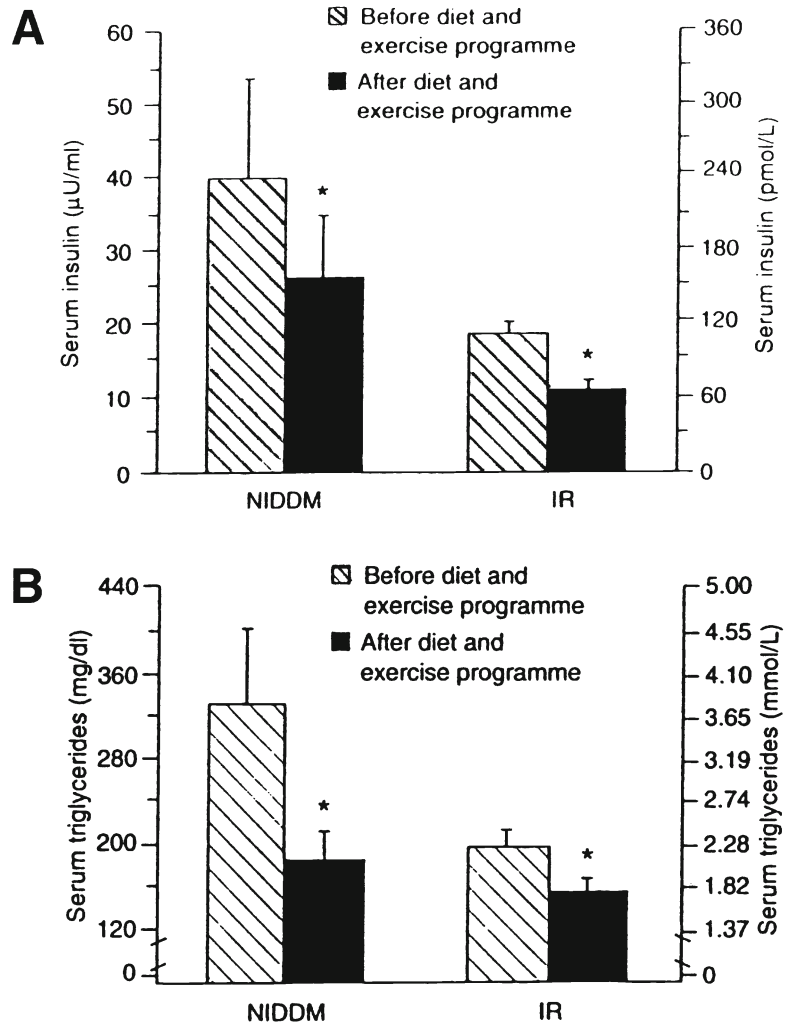

Fig. 6. (**A**,**B**), Effects of high-complex carbohydrate diet and daily aerobic exercise on s-insulin and s-triglyceride level. (From ref. *146.*)

however, increases in HDL-C and decreases in very-low-density lipoprotein cholesterol, triglycerides, and blood pressure are thought to be contributing factors. Information on the effect of physical activity and physical fitness on obesity and CVD risk indicators in children and adolescents indicate that a high level of physical activity and physical fitness have a favorable effect on serum lipids and serum insulin concentrations and obesity *(143,153–155).*

6.4. Sociocultural Environment

Similar to many other lifestyle-related diseases (e.g., smoking), obesity first appeared in the affluent parts of the society and was regarded as a sign of health and prosperity. With increasing awareness and knowledge of the negative health consequences, more well-educated groups with higher socioeconomic status (SES) have adopted more

healthy dietary and physical activity habits, thus counteracting the development of obesity. Therefore, obesity is becoming more prevalent in lower socioeconomic groups *(99)*. Easy access to inexpensive, energy-dense food has contributed to this development *(156)*. To varying degrees, obesity is a social handicap and a downward social mobility of obese individuals could contribute to the higher prevalence of obesity in lower socioeconomic groups *(157)*. A parallel development is seen in CVD mortality, where the decline in mortality during the last decades has not occurred in the lower social classes, resulting in widening social health inequality *(158)*.

Psychosocial stress may be another link between social environment and obesity. Neuroendocrine responses to psychosocial pressures, especially the defeat reaction, are well-established, with increased activity of the hypothalamic–pituitary–adrenal axis and increased cortisol and inhibition of gonadotropin secretion. Such endocrine disturbances are followed by metabolic aberrations and probably also by the accumulation of visceral fat *(159)*.

In Western societies, low SES and educational level of the parents is related to obesity in children *(99)*, whereas in developing countries, children in affluent families are more at risk. Urbanization appears to be an important factor *(160)*. The mechanism behind this association is probably a combined effect of dietary and physical activity habits, cognitive stimulation, and economic limitations for a healthy lifestyle *(161,162)*. Low-income mothers do not perceive weight problems in their children negatively and are inclined to think that the weight problem is inherited and not influenced by environmental factors *(163)*.

7. TREATMENT AND PREVENTION

Childhood weight problems have long been regarded as a rather benign condition with a favorable prognosis even without intervention for the majority of the children, making health professionals rather reluctant and passive when confronted with the overweight child. The focus in health surveillance and dietary advice and recommendations to parents has been on preventing malnutrition to ensure the children eat as much as possible. This is also inherited in parenthood, as indicated by phrases such as. “Be a good boy/girl and clear your plate.” The epidemiological development during recent decades indicates that the focus must be changed by health professionals, parents, and society.

7.1. Treatment

Although it is well-documented that it is possible to reduce obesity by modifying energy intake and expenditure, treatment of manifest obesity in both adults and children has been disappointing, with very few programs showing lasting weight reduction *(164–168)*. Even if it seems logical that interventions during childhood would be easier, there is only some evidence to support this notion *(169)*. Pharmacological and surgical therapies have limited value, especially in children and adolescents, and should be used exclusively for severe cases in a clinical situation *(170)*. Family- or school-based interventions focusing on changing diet and physical activity are the two major approaches used *(166)*. It has become increasingly clear that information alone is not enough to change dietary and physical activity habits *(171)*. Eating habits and physical activity are two basic human behaviors that are not easily changed. By nature, humans are genetically

driven to move no more than necessary and to eat as much as possible when food is available. There seems to be no "defense" against overeating and a sedentary life *(81)*. Therefore, changing and controlling these behaviors requires a voluntary determination and willpower. As a consequence, behavioral strategies and techniques are used in intervention programs. Some specific techniques that seem important include contracting, self-monitoring, social reinforcement, and modeling *(165)*. In younger children, parental involvement is crucial, focusing more on changing parental guidance behavior than changing behavior of the individual child. A prerequisite for successful treatment of obesity in children is a strongly motivated child and family *(172)*. A family history of obesity is an important argument for intervention. However, as the risk of obesity gradually increases *(16)*, the optimal timing and content of the intervention must be evaluated further.

Concern has been raised that dieting programs could have negative side effects on growth and might also provoke eating disorders, but there seems to be little evidence of these complications *(135,165,173)*. However, caution is recommended when giving dieting advice during midadolescence, because some adolescents—mostly girls—are very much concerned with their body weight and may be too responsive to advice about low-fat food and exercise. As part of an anorectic behavior, some of these girls already have low intake of energy and fat, and further reduction could have a negative influence on their growth and health *(174)*.

7.2. Prevention

The size of the problem and the poor outcome of treatment imply that prevention is the only feasible way to cope with the obesity epidemic. Preventive measures may target individuals, groups, or the whole society and environment *(166,175–177)*, and the focus of prevention is the same as in treatment, that is, controlling energy intake and expenditure. Primary prevention (preventing obesity development) is, of course, the ultimate goal, but the first step is to try to control the epidemic by stopping the increase. Secondary prevention with early identification of children at risk and proper intervention is also of great importance. The introduction of BMI curves and standardized cut-off values for classifying obesity in children and adolescents gives health professionals a new screening tool for identifying children at risk *(58)*.

At least in theory, schools are a suitable setting to target children and adolescents by encouraging physical activity and other interventions aimed at preventing obesity. Preventive measures for all children may also act as treatment for already obese children and can also minimize the stigmatization. However, school intervention programs have been as discouraging as treatment programs. Using health education, the school programs have been trying to decrease fat consumption, increase consumption of fruits and vegetables, promote physical activity, and limit sedentary activities (e.g., television viewing). Despite intensive broad-scaled efforts most interventions have failed to reduce obesity prevalence, although some behavioral changes have been observed *(178)*. One lesson learned, which confirms the notion that eating and physical activity behaviors are more difficult to change as the children get older, is that strategies aimed at younger children seem to have better long-term effects than those focused at adolescents *(175,178)*.

However, it should, be emphasized that it is not sufficient to tackle the obesity problem only as behavioral problem (i.e., teaching people to control their eating and activity behavior). The obesogenic society and environment must also be changed, requiring

coordinated efforts from governments and the private sector *(179–181)*. Several environmental factors in modern society promote excess energy intake and limit energy expenditure in children, undermining individual efforts to maintain a healthy body weight. Meals eaten away from home in the United States have nearly doubled in the past decade, with a parallel profound increase in fast food and soft drink consumption. Inexpensive fast food of increasing portion sizes is readily available compared to healthy food choices such as fruits and vegetables. Fast food and sweets also are available in vending machines and school cafeterias. The result of this is an overconsumption of sugar and saturated fat and an underconsumption of fruits and starchy vegetables, fiber, and some micronutrients. An important factor behind these dietary changes is that the food industry has invested huge sums of money on advertising and marketing campaigns that directly target children and their parents *(111,166,182)*.

In addition to dietary changes, children and adolescents are becoming more sedentary, spending increasingly more time with computers and videos and watching television. Physical education at school has decreased and traffic in the cities has made the environment too dangerous for children to move around and play outdoors on their own after school. Children are brought to and picked up from school and leisure activities by car or bus. The drive-through window of fast food restaurants, where a maximum of energy can be obtained with minimum effort, is the "image" of the obesity problem *(166)*.

What can be done? Perhaps lessons can be learned from epidemiology and public health interventions against other epidemics in children (e.g., infectious diseases and injuries). In many countries, these epidemics are currently controlled by a combination of individual and environmental programs that increase parental and child knowledge, skills, and risk awareness and environmental programs that reduce the exposure of the "noxious agent." For obesity, this implies educational and counseling programs that increase parental knowledge and skills about nutrition and risk awareness of obesity *(17,183,184)* combined with governmental nutritional programs (e.g., in schools; ref. *185*) that support healthy diets and physical activity *(180)*.

8. SUMMARY AND RECOMMENDATIONS

Obesity has been classified as a modern epidemic, evolving during recent decades in both industrialized and developing countries. The epidemic also includes children and adolescents. Obesity is related to increased morbidity and mortality in a number of diseases, most importantly NIDDM and CVD. The clinical entity of obesity, hyperglycemia, dyslipidemia, and hypertension, which is also seen in children and adolescents, is referred to as the "metabolic syndrome." Increased resistance to insulin in liver, muscle, and adipose tissue is regarded as an important explanation behind the disturbances in glucose and lipid metabolism. IR is closely related to obesity, and visceral fat accumulation seems to have a particularly harmful effect. Although genetic and early nutritional factors may influence the susceptibility to weight gain, the major underlying cause of the obesity epidemic is the spread of a sedentary Western lifestyle (i.e., a combination of low physical activity and consumption of high-fat, high-sugar, and high-animal protein diets, with energy intake exceeding energy expenditure). Attempts to achieve lasting weight reductions in obese adults have been disappointing. Although it may be easier to treat obesity in children than in adults, it is obvious that the best strategy is primary prevention targeting all children. However, as obesity can be regarded as an epidemic caused by modern lifestyle,

effective preventive measures must not focus only on individual behavior but also on the social and physical environment for children, supporting more daily physical activities (not only sports) and healthy diets. This will not be an easy task, because there are a number of factors operating in the opposite direction, including: motorization; restricting physical activities both for convenience and safety reasons; pursuit of sedentary recreations during leisure time, such as computers, video games, and television; and easy access to high-fat/-sugar fast foods. Improving parents' nutritional knowledge and awareness of the risk of obesity through educational programs and counseling could be a way of achieving a useful "immunization program." However, it is essential that the entire society be changed to be more sensitive to children and their future health.

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13 Prevention of Pediatric Obesity

Examining the Issues and Forecasting Research Directions

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KEY POINTS

- The prevalence of childhood obesity has dramatically increased during the past 20 to 30 yr, paralleling the trends seen in adults.
- Childhood obesity is associated with hypertension, hyperinsulinemia, glucose intolerance, type 2 diabetes, dislipidemias, and other comorbidities.
- Identifying specific environmental contributors to childhood obesity may help to guide the development of novel prevention strategies.
- School-based programs targeting reduced television viewing have been shown to help prevent weight gain in children.
- Coordinated efforts between the food industry and government policy changes may be at the forefront of future obesity prevention efforts.

1. INTRODUCTION

The obese community is taking a strong stance on the prevention of childhood obesity. (We use the term "obesity" in this chapter, but acknowledge Centers for Disease Control and Prevention's definitions of childhood "overweight" as any body mass index [kg/m^2] equal to or exceeding the age-sex-specific 95th percentile for BMI, and "at risk for overweight" as any BMI between the 85th and 95th percentiles.) Unlike other issues for which there is less consensus, obesity and other health researchers have placed obesity prevention at the top of the research agenda. Given the increasing prevalence of pediatric obesity in the United States and its pervasive health and economical costs *(1,2)*, various health organizations have clearly prioritized research to better understand the causes of obesity and to promote the development of innovative prevention strategies *(3,4)*. This interest is exemplified by the Healthy People 2010 Objectives, which

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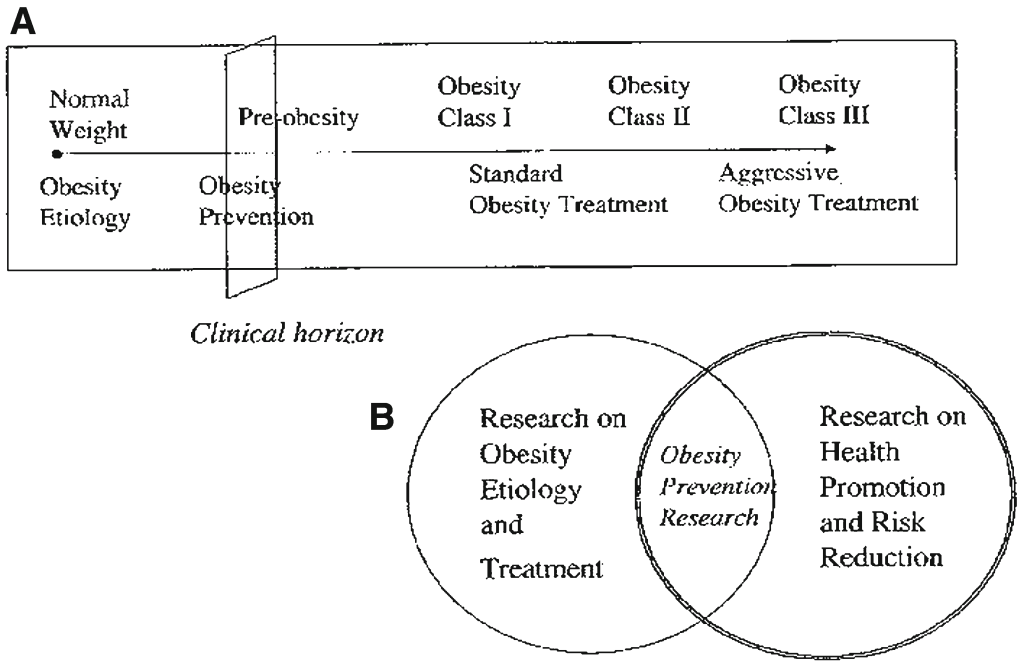

Fig. 1. Views of obesity prevention and research from the NIH "Pathways to Prevention" Workshop. **(A)** depicts a framework in which prevention is essentially conceptualized as treatment of subclinical obesity. **(B)** depicts a framework in which prevention is conceptualized as combining knowledge from the obesity research field with knowledge and strategies from the broader field of health promotion. (From ref. *7*.)

aim to decrease the prevalence of excessive weight among adolescents from 11% (the rate in 1988–1994) to 5% by 2010 *(5)*.

In an important position paper in 1994, the National Institute of Diabetes and Digestive and Kidney Diseases' (NIDDK) National Task Force on Prevention and Treatment of Obesity prepared a call to action, which called the prevention of obesity "the most compelling new research need in the obesity field today" *(6)*. Other prominent scientific bodies, including the Institute of Medicine at the National Academy of Sciences, also called for more research into the prevention of obesity *(4)*. Most recently, a National Institutes of Health (NIH) workshop, "Pathways to Obesity Prevention," summarized the interim findings of 20 ongoing pilot obesity prevention studies targeting children and adults *(7)*. This workshop concluded that the existing "treatment" paradigm may not be the optimal model for prevention and may need to bridge conceptual frameworks from the fields of basic science, health promotion, and risk reduction. Figure 1 highlights this framework.

This chapter examines issues in the prevention of childhood, rather than adulthood, obesity. Therefore, prevention efforts involving worksite programs or large-scale community efforts targeting adults are not included. Detailed discussions on these programs are available elsewhere *(8–10)*. The chapter is broadly divided into four sections. The first section discusses why the prevention of childhood obesity is a high priority. The second section reviews causal mechanisms of childhood obesity, with attention to environmental factors that may have contributed to the recent increases in prevalence of

childhood obesity. The third section reviews existing programs for the prevention of childhood obesity as well as their clinical efficacy. Finally, the fourth section examines theoretical issues for future prevention research regarding children and adolescents. We conclude by reviewing practical clinical recommendations from the American Academy of Pediatrics. Please also *see* Chapters 12 and 14.

2. PRIORITIZING THE PREVENTION OF CHILDHOOD OBESITY

2.1. Increasing Prevalence of Childhood Obesity

Broadly speaking, obesity can be defined as an excess of adiposity. Although adipose tissue cannot be directly measured in living humans, various indirect measures are available for use with children and adolescents *(11,12)*. These measures include the BMI *(13)*, weight-for-stature index *(13)*, skinfold measures *(14)*, bioelectrical impedance analysis *(15)*, and dual-energy X-ray absorptiometry *(14–16)*. More detailed descriptions of the advantages and limitations of these methodologies are provided elsewhere *(11,12,17)*. The most recent data on the prevalence of childhood obesity comes from the fourth National Health and Nutrition Examination Survey, a nationally representative survey of the US civilian, noninstitutionalized population. "At risk for overweight" was defined as any BMI-for-age score between the 85th and 95th percentiles; using scores from the 1970s as the reference comparison group, 25% of all children ages 6 to 19 yr who were measured between 1999 and 2000 were classified as "at risk for overweight." The prevalence of "overweight," defined as any BMI score greater than or equal to the 95th percentile, was 15.5% among 12- to 19-yr-olds, 15.3% among 6- to 11-yr-olds, and 10.4% among 2- to 5-yr-olds. Comparable estimates from 1988 to 1994 were 10.5, 11.3, and 7.2%, respectively.

The rise in prevalence of obesity has been particularly prominent among non-Hispanic African-American and Mexican-American adolescents. The prevalence in these groups increased more than 10% between the years 1988–1994 and 1999–2000, with more than 23% of non-Hispanic African-American and Mexican-American adolescents being classified as overweight in 1999–2000. This prevalence increased 13% among Mexican-American males during this period. Among males evaluated in 1999–2000, the prevalence of being overweight was 12.8% for non-Hispanic Caucasian adolescents, 20.7% for non-Hispanic African-American adolescents, and 27.5% for Mexican-American adolescents.

Studies that have used skinfold measures to assess subcutaneous body fat have also documented an increased prevalence of childhood and adolescent obesity over the past several decades *(18–24)*. Kuczmarski *(23)* studied changes in the median value of triceps skinfold thickness between 1963–1965 and 1976–1980. He noted a substantial increase in the median triceps skinfold measurement over time, particularly among males ages 6 to 11 yr and females ages 12 to 17 yr. Changes were less notable for children younger than age 6 yr. Also noteworthy was the increasing skinfold thickness corresponding to the 95th percentile of the population. This increase was greatest for males ages 6 to 11 yr.

A more recent study by McCarthy et al. *(25)* examined changes in waist circumferences in British children ages 11 to 16 yr. Using data from national cross-sectional nutritional surveys administered in 1977, 1987, and 1997, they found that the average waist circumference score sharply increased across the years. Specifically, the mean increased by 6.9 ± 0.84 cm and $6.2 + 1.02$ cm for males and females, respectively. By

1997, 28% of the males and 38% of the females had waist circumference scores that exceeded the 91st percentiles that existed for boys and girls in 1977–1978.

3. COMORBIDITIES IN CHILDHOOD

A mounting number of studies have documented the health risks of pediatric obesity. These risks include elevated blood pressure, glucose intolerance, hyperinsulinimia, dyslipidemias, and type 2 diabetes *(26)*.

3.1. Blood Pressure

Several studies have documented an association between adiposity and elevated blood pressure among pediatric and adult samples *(27–30)*. For example, the Muscatine study of adolescents and young adults found that hypertension was twice as prevalent among overweight than normal-weight subjects *(29)*. Another study examined data from more than 3000 males and females ages 5 to 18 yr *(30)* and found that fatness in excess of 25 and 30% of total body mass in males and in females, respectively, was associated with increased risk for hypertension. Similar findings were also noted among 5- and 6-yr-old Hispanic children *(31)*. A recent study also found that compared to nonobese adolescents, obese adolescents had greater cardiac mass and increased isovolumic relaxation time, which are indicative of increased heart work *(32)*. Recently, Daniels et al. *(33)* examined the predictors of left atrial enlargement in children and adolescents with essential hypertension. This condition is especially concerning given its association with cardiovascular disease and stroke in adults. In a sample of 112 adolescents, BMI was significantly elevated among participants with left atrial enlargement compared to those without the condition.

3.2. Type 2 Diabetes Mellitus, Hyperinsulinemia, and Glucose Intolerance

Body fatness is a reliable correlate of hyperinsulinemia and glucose intolerance among adolescent males and females *(34–38)*. It has been proposed that hyperinsulinemia may underlie the association of obesity, hypertension, and glucose intolerance *(39)*. Although most studies have used adult subjects, there are supportive data from pediatric samples *(37,40)*. To the extent of accuracy in the model, possible mechanisms include increased sodium retention, stimulation of the central nervous system, and abnormal vascular regulation *(32)*.

Impaired glucose tolerance (IGT) is a condition that is indicated by elevated blood sugar levels (but not to levels high enough for a diagnosis of type 2 diabetes). A clinic-based study found IGT in 25% of obese children ages 4 to 11 yr and 21% of obese children ages 11 to 18 yr *(41)*. Moreover, 14 girls in the sample had polycystic ovary syndrome (PCOS)—a disorder that is associated with menstrual irregularities, hirsutism caused by high androgen levels, infertility, and cysts on the ovaries. Compared to obese girls without PCOS, obese girls with POS are more likely to have metabolic derangements that include insulin resistance (IR) *(42)*.

IR, another precursor to type 2 diabetes, is also related to childhood obesity. *(43)*. In a school-based study of more than 12,000 boys and girls in the fifth to eighth grades, Sinaiko et al. *(44)* found significant associations between IR and body fat. The authors found evidence for increased body fat, elevated trigylcerides and low-density lipoprotein (LDL) cholesterol, reduced high-density lipoprotein (HDL) cholesterol, and elevated

blood pressure among the most insulin-resistant children. This suggests the potential emergence of a "metabolic syndrome" during childhood.

Pinhas-Hamel and associates *(45)* reported a striking increase in the number of children diagnosed with type 2 diabetes mellitus in the primary care setting. In an analysis of 1027 children and adolescents attending a Cincinatti clinic, only 4% were diagnosed with type 2 diabetes mellitus before 1992. By 1994, 16% of new cases of type 2 diabetes were diagnosed in that age group, with 10- to 19-yr-olds representing 33% of those cases. This translated into a 10-fold increase in the incidence of diabetes within the 10- to 19-yr age group. Within this sample, risk factors for a positive diagnosis included family history, ethnicity (greater risk in African-Americans), and excessive weight *(45)*.

3.3. Lipids and Lipoproteins

Pediatric obesity is associated with elevated levels of total cholesterol, LDL cholesterol, and triglycerides and decreased levels of HDL cholesterol *(46–49)*. Furthermore, there appears to be an association between central adiposity and adverse lipid profiles that is independent of total body fat *(50)*. This association is more pronounced among individuals who have already begun sexual development and have moderate-to-high levels of total body fat *(50)*. Other studies measuring body composition have produced comparable findings regarding children's lipid profiles *(51,52)*.

Using nuclear magnetic resonance spectroscopy, Freedman et al. *(53)* tested the correlates of LDL and very low-density lipoprotein (VLDL) in 10- to 17-yr-olds from the Bogalusa Heart Study. They found that levels of triglycerides, insulin, and relative weight were positively associated with the particle size of VLDL and were negatively associated with LDL particle size. Children who were at risk for excessive weight or who were overweight were 2.4 times as likely as normal-weight children to have elevated total cholesterol, diastolic blood pressure, LDL cholesterol, systolic blood pressure, triglycerides, and fasting insulin. Several of these associations differed between Caucasians and Afircan-Americans and by age.

3.4. Respiratory Abnormalities

In non-obese individuals, there is usually little fat deposition in the neck and in the peripharynx regions. Increased neck adiposity has been observed among obese adult subjects and is associated with respiratory abnormalities, such as hyperventilation and obstructive apnea *(54)*. Similarly, increased neck adiposity is associated with respiratory abnormalities during sleep among pediatric samples. Examples of conditions that interfere with sleep include subclinical nocturnal desaturation or decreased oxygen saturation (<90%) in red blood cells *(55)* and adenoidal tonsillar hypertrophy *(56)*. Respiratory complications associated with childhood obesity include asthma, obstructive sleep apnea, and Pickwickian syndrome *(57,58)*.

3.5. Pseudogynecomastia

Pseudogynecomastia is excessive deposition of fat in the chest; it is particularly prevalent during adolescence. Although pseudogynecomastia is of minimal health risk, our clinical experience suggests that it has negative effects on body image among obese male adolescents. This is an important issue in light of evidence that obesity is related to dissatisfaction with body image *(59,60)*.

3.6. Nonalcoholic Fatty Liver Disease

Pediatric nonalcoholic fatty liver disease, characterized by accumulation of fat in the liver, is becoming more common as the prevalence of childhood obesity increases. This disorder typically is associated with obesity, diabetes, hepatotoxicity, and certain genetic/metabolic diseases in adults. Until recently, it was considered to be rare in children; however, its occurrence has been linked to the rapid rise in childhood weight. The disorder is more common among males and may present in children as young as age 4 yr *(61,62)*.

3.7. Social Stigma and Depression

The psychosocial stressors and stigmatization of obesity can be very concerning for many obese children. Strauss and Pollack *(63)* recently investigated the social networks of more than 17,000 overweight and normal-weight adolescents (age range: 13–18 yr) enrolled in the National Longitudinal Study of Adolescent Health. They compared the two groups and reported fewer friendship nominations and more peripheral peer relations among overweight, rather than normal-weight, adolescents.

Recent studies have suggested a possible association between obesity and depression *(64,65)*, including those studies of adolescent samples *(65)*. Erikson et al. *(66)* found that BMI was significantly positively associated with depression levels in adolescent girls ($r = 0.14$; $p < 0.01$) but not in boys ($r = 0.01$; $p < 0.78$) in more than 800 children from 13 California schools. Eisenberg et al. *(67)* found that history of weight-teasing by peers was associated with elevated depression, body-image disparagement, and suicidal ideation and attempts.

3.8. Mortality Rate

Finally, several longitudinal studies have assessed the long-term impact of childhood obesity on mortality rate and morbidity *(68–71)*. For example, Must and colleagues *(69)* used 55-yr of follow-up data to evaluate the relative risks of mortality associated with obesity during adolescence. Among males, the relative risks for all-cause mortality and atherosclerotic cerebrovascular disease were 1.8 and 13.2, respectively. The effect of BMI during adolescence on all-cause mortality among males appeared to independent of adulthood BMI. Among females, however, the association between childhood obesity and adult mortality was not statistically significant. Such findings suggest that among males, the health effects of childhood obesity may not be short-term and may persist into later life.

4. MECHANISMS OF CHILDHOOD OBESITY

4.1. Genetic Influences

It has been suggested that the acorns do not fall far from the oak tree, implying that children grow up to be quite similar to their parents. Similarly, it is well-established that individuals within families tend to be similar regarding their obtained degree of fatness. This intrafamily similarity could be a result of either genetic influences or a shared common environment. Among animal breeders, it has long been known that selective breeding for fatness and leanness is possible, indicating a strong genetic influence on adiposity in domestic animals *(72)*. Among humans, there now is additional substantial evidence that genetic factors exert a strong influence on adiposity *(73,74)*. For most

individuals in the population, obesity is a polygenic disorder that results from the interplay of many genes. However, the topic of genetic influences on childhood obesity is very complicated and well beyond the scope of this chapter. Reviews on the association of genetics and childhood obesity are provided elsewhere *(75–77)*. One potential avenue for future research may be targeting children who are identified as having "obesity-promoting genes" for prevention strategies. Such possibilities may be unlikely in the immediate future but are not inconceivable given the pace of contemporary molecular genetics research.

4.2. Environmental Influences

Obesity is partly an environmentally influenced disorder. Identifying the specific environmental factors that promote obesity onset may help guide the development of more effective prevention strategies. This section reviews data on environmental predictors of childhood obesity.

First, there is a small, but positive, association between socioeconomic status (SES) and obesity among children living in industrialized societies, although this trend may change during adolescence *(78)*. There is some evidence that girls from low-income families become heavier after adolescence, whereas girls from high-income families become leaner after adolescence *(79)*. Indeed, among adult females, there is a negative association between SES and adiposity *(78)*. Second, there is correlational evidence that family discord is associated with increased BMI among children and adolescents *(80,81)*. A European study conducted a 10-yr follow-up of children who apparently were either "neglected" or "not neglected" by their parents *(82)*. Neglected children were 9.8 times more likely to become obese adolescents than non-neglected children, suggesting that environmental stressors might contribute to obesity development.

Third, there is strong evidence that television viewing is associated with obesity among children *(83–85)*. Increased viewing hours may lead to obesity by displacing energy expenditure of physical activity *(86)*, although other factors related to dietary intake also are likely *(87)*. One noteworthy study compared the frequency of sports participation among children living in three towns with differential exposure to television. Compared to children living in towns with television, those living in a town without television participated in more sports *(88)*.

Fourth, larger portion sizes of foods may promote increased body weight in children. A study of marketplace foods by Young and Nestle *(89)* found that the portion sizes of virtually all foods sampled have increased significantly since the 1970s, thus exceeding federal dietary guidelines on a consistent basis. Results are shown in Fig. 2. The largest increase according to US Department of Agriculture (USDA) standards occurred in the cookie category, the size of which was 700% greater than USDA recommendations *(89)*. Rolls et al. *(90)* conducted an experiment in which they served preschool children small (150–225 g), medium (263–338 g), and large (376–450 g) portions of a main course entrée at three different lunches. Results indicated that older children (5 yr), but not younger children (3 yr), ate more food when exposed to lunches that were larger in portion sizes *(90)*. These results are depicted in Fig. 3. The results suggest that children may become more responsive to external cues as they age, which may increase susceptibility to obesity.

Finally, it has been suggested that restrictive feeding practices may promote childhood obesity by impeding a child's ability to recognize internal feelings of hunger and

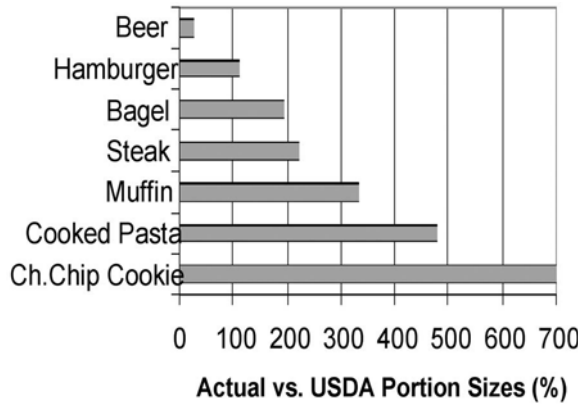

Fig. 2. Actual portion sizes vs USDA portion sizes of seven common foods. (From ref. *89*.)

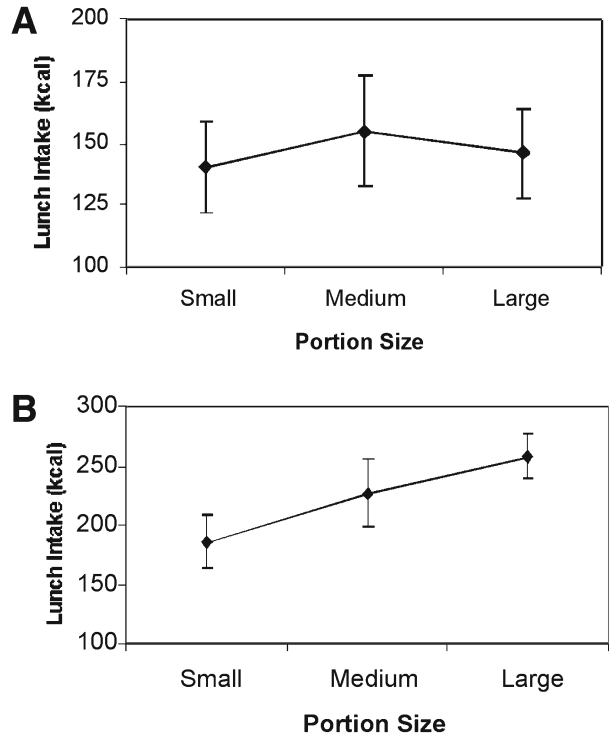

Fig. 3. The effects of meal portion sizes on total energy intake at lunch in 3-yr-old children **(A)** and 5-yr old children **(B)**. (From ref. *90*.)

satiety. A classic study *(91)* found that children whose parents had a more controlling style of feeding were less adept at self-regulating the calories they consumed in a laboratory setting. Compared to children whose parents had a less controlling feeding style, children whose parents had a more controlling feeding style tended to over consume calories at an experimental lunch meal that followed a liquid "preload" beverage. These

Table 1
Expert Panel Recommendations for Parenting Guidelines for Pediatric Obesity Treatment

- Find reasons to praise the child's behavior.
- Never use food as a reward.
- Parents can ask for "rewards" for children in exchange for the changes in their own behavior.
- Establish daily family meal and snack times.
- Parents or caregivers should determine what food is offered and when, and the child should decide whether to eat.
- Offer only healthy options.
- Remove temptations.
- Be a role model.
- Be consistent.

From ref. *17*.

children were poorer at so-called "caloric compensation," which in turn was associated with increased body fat measures in girls. Restrictive feeding practices by parents also have been associated with elevated "eating in the absence of hunger" by children—that is, the tendency to snack on foods after completing a meal and despite feeling full *(92)*. Eating in the absence of hunger stablizes in children over time *(93)* and was shown in one prospective study to predict increased body weight in girls *(94)*. On the other hand, disentangling cause-and-effect is a challenge for such studies, because parental feeding restriction may be responsive to a child's eating and weight characteristics *(95)*. This remains a very active area of scientific investigation, because it has important implications for caregiver-based obesity prevention strategies. Table 1 presents a list of practical recommendation from an expert panel regarding parenting guidelines for parents of obese children *(17)*.

5. PREVENTION OF CHILDHOOD OBESITY

Public health researchers traditionally have described three types of prevention: primary prevention, secondary prevention, and tertiary prevention *(4)*. Primary prevention is the closest concept to true prevention, in which a medical condition is prevented through early and aggressive intervention. Ideally, intervention completely preempts or interrupts the emergence of the condition even before its early stages. Thus, the primary prevention of pediatric obesity would require early-life detection of excessive adiposity or the identification of genetic markers to target individuals who would probably become obese if left untreated. Once adiposity or genetic markers are identified, primary preventive services are aggressively introduced to circumvent the emergence of obesity.

Secondary prevention refers to early intervention soon after signs of a condition emerge, although this intervention does not occur as immediately as primary prevention. For example, the secondary prevention of pediatric obesity entails prompt intervention services soon after a child is assessed as being obese. Some believe that elementary school personnel could be effective and practical agents of secondary prevention services *(96)*. However, as discussed later, existent data on this topic are mixed.

Finally, tertiary prevention refers to intervention after a condition has manifested itself for a period of time. For example, intervention with a 15-yr-old obese teenager

who has been overweight for his or her entire life is an example of tertiary prevention. The encouraging outcomes of several prominent behavioral interventions for pediatric obesity are examples of tertiary intervention *(97,98)*. Ironically, the phrase "tertiary prevention" may be a misnomer because the goal is not actually to prevent but, rather, to treat an existent disease *(4)*. On the other hand, the tertiary prevention of childhood obesity can be conceptualized as the primary prevention of adulthood obesity *(99)*.

There has been surprisingly little research on the prevention of pediatric obesity despite its increasing prevalence and associated health risks. Still, there have been attempts to prevent pediatric obesity at the primary, secondary, and tertiary levels *(100–103)*.

5.1. Primary Prevention Studies

To our knowledge, there is only one controlled primary prevention study in the literature that targeted infants *(104)*. In this study, the researchers used nutrition education for the parents of 80 neonates. These parents received a nutritional guide, "The Prudent Diet," to guide food preparation for the children once they reached age 3 mo and a weight of approx 6 kg. This guide provided very specific recommendations for food selections, portion sizes, and calorie content. The parents of 50 control infants received instructions on a "conventional" diet for their infants. At a 3-yr follow-up, the data clearly indicated the effectiveness of the intervention. Among female subjects measured after 3 yr, 16.7% of the control infants were overweight compared to no overweight infants in the experimental group. Among male subjects, 34.8% of the control group was overweight compared to 2.56% of the experimental group.

Fitzgibbon and colleagues *(105)* described an obesity prevention pilot program for African-American mothers and their pre-adolescent daughters. Their report described a 6-wk controlled intervention study, with treated families receiving what was described as an education- and skills-based approach for managing health behaviors. Compared to control subjects, there was a nonsignificant tendency for mothers and daughters in the experimental group to report a decrease in daily fat intake. Mothers and daughters in the experimental groups also reported increased nutritional knowledge compared to control subjects. Changes in BMI or other indexes of fatness were not reported.

In an intervention designed to prevent adulthood obesity, Epstein et al. *(106)* conducted a randomized controlled trial with 8- to 10-yr-old children who had at least one obese parent but were not yet obese themselves. Families in one group received a behavioral intervention that explicitly targeted increased fruit and vegetable intake, whereas families in the second group received a behavioral intervention that explicitly targeted reduced dietary fat intake. Results indicated that children in both groups showed significant increases in fruit and vegetable intake and reductions in percentage of excess weight over 12 mo.

5.2. Secondary and Tertiary Prevention Studies

5.2.1. School-Based Interventions

There have been a variety of school-based programs, some of which were school-wide, that have targeted both obese and non-obese children *(107–109)*, whereas others have specifically targeted obese children *(110,111)*. These programs tended to be multifaceted, including nutrition education and school-based exercise components. Stimulus-control techniques, self-monitoring, and problem solving were components of these programs.

Resnicow *(96)* reviewed six controlled interventions that specifically targeted overweight children. With interventions lasting from 12 wk to 18 mo, children in the treatment groups gained significantly less weight over time than control subjects in five out of the six studies. Despite these findings, numerous issues restrict enthusiasm. As Resnicow noted, subjects were not always randomly assigned to groups. Second, moderators of treatment outcome have not been systematically addressed across studies. Finally, there are few long-term follow-up data on children undergoing such programs, and the available data are not encouraging *(111)*.

The findings from one of the largest and most comprehensive school-based health interventions to date, the Child and Adolescent Trial for Cardiovascular Health, are especially noteworthy *(112)*. This controlled intervention studied more than 5000 ethnically diverse children from 96 schools in California, Louisiana, Minnesota, and Texas. Despite the magnitude of this program, 3-yr health outcomes on a variety of measures, including BMI and skinfolds, were disappointing. Significant changes were primarily noted on self-reported measures of food intake and exercise.

Two of the most influential school-based prevention studies in recent years were those by Robinson *(113)* and Gortmaker et al. *(114)*. Robinson's school-based study compared 192 third- and fourth-grade students (mean age: 8.9 yr) from two California elementary schools. The intervention of the study consisted of an 18-lesson, 6-mo classroom curriculum to reduce television, videotape, and video game use. Compared to the control school, the treatment school demonstrated less of an increase in BMI (intervention vs control changes: from 18.38 to 18.67 vs from 18.10 to 18.81 kg/m^2), less of an increase in triceps skinfold measurements (intervention vs control change: from 14.55 to 15.47 vs from 13.97 to 16.46 mm), less of an increase in waist-to-hip ratio (intervention vs control change: from 0.83 to 0.83 vs from 0.82 to 0.84), and less of an increase in waist circumference (intervention vs control change: from 60.48 to 63.57 cm vs from 59.51 to 64.73 cm). The intervention group also demonstrated statistically significant decreases in reported television viewing (intervention vs control change: from 15.35 to 8.80 vs from 15.36 to 14.46 h/wk). This study provided a strong illustration of the potential role of reduction of television viewing in obesity prevention.

Gortmaker et al.'s *(114)* "Planet Health" obesity prevention program also emphasized reduction of television viewing as a central component in their obesity prevention program. The researchers compared five treatment schools against five control schools in a study that included 1,295 ethnically diverse children in grades 6 and 7 in Massachusetts. Over 2 yr, the obesity prevalence among girls in the intervention schools declined from 23.6 to 20.3%, whereas the prevalence among girls in the control schools increased from 21.5 to 23.7%. Moreover, changes in television viewing practices predicted treatment outcome for girls. Each hour of reduced television watching was independently associated with a 15% reduction in the likelihood of obesity (odds ratio = 0.85; $p = 0.02$). It also should be noted that the control and treatment schools did not differ regarding obesity incidence (i.e., the number of new obesity cases over time).

5.2.2. Behavioral Weight-Loss Interventions

In contrast to the discouraging results for adults *(115)*, family-based behavioral interventions can promote long-term weight loss in obese children *(97,98,116,117)*. These promising findings primarily come from Epstein's research program, which includes diet modification and exercise promotion. This program has resulted in sustained

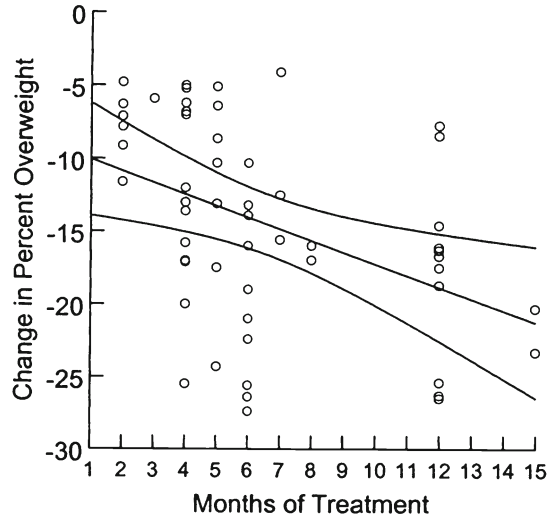

Fig. 4. Association between months of family-based behavioral treatment for childhood obesity and change percent of excess weight in child: results of 39-study meta-analysis. (From ref. *119*.)

weight loss even at 10-yr of follow-up *(98)*. Although these findings do not qualify as primary prevention, they provide important insights into variables that might moderate the efficacy of primary prevention programs. For example, the greatest reductions in percentage of excess weight at 10-yr of follow-up were found in treatments incorporating parental involvement in treatment as well as activity-promoting lifestyle changes or aerobic exercise, rather than calisthenics *(98)*. In another study, the greatest reductions in percentage of excess weight and percentage of body fat were associated with treatments targeting the reduction of sedentary behavior rather than exercise promotion or a combination of the two *(118)*.

In a meta-analysis of 39 family-based behavioral interventions that targeted dietary and/or physical activity modification, Goldfield et al. *(119)* demonstrated a relationship between treatment duration and reduction in child overweight percentage (*see* Fig. 4). Using multiple regression techniques, they demonstrated that children treated for 15 mo would be expected to reduce their percentage of excess weight by approx 20% on average.

6. APPROACHES TO PREVENTION: THEORETICAL PERSPECTIVES

There are different strategies for approaching the prevention of pediatric obesity. Four dimensions of prevention effort research include targeting, philosophy, punitiveness, and timing.

6.1. Target of Prevention Efforts: "Shotgun" vs "Rifle" Approaches

One fundamental consideration for prevention research is the target of intervention—that is, prevention efforts can target entire populations or specific individuals within the population (i.e., high-risk individuals). The former, a "shotgun" approach, strives to reach most people with modest impact, whereas the latter, a "rifle" approach, strives to aggressively intervene with an at-risk subgroup. The shotgun approach might use psycho-educational

approaches, including information dissemination via mass media and community activism, to reach most individuals within a community. Such strategies have traditionally been used in community prevention efforts with adults as well as many school-based programs with children.

In the "rifle" approach, aggressive prevention services depend on prescreening children for obesity or markers for future obesity. For example, given the high heritability of obesity, prevention efforts might target children whose parents and other family members are obese. An expert panel proposed a two-level screening protocol for determining those adolescents that might be most appropriate for prevention service *(120)*. The first level of these guidelines is a BMI exceeding the 95th percentile for a given age and gender or a BMI greater than 30—whichever is smaller. The second level assesses the following four criteria: elevated blood pressure, total cholesterol, large increments in BMI, and concern by the adolescent about his or her weight. Any adolescent who meets the first-level criterion and any of the second-level criteria would be considered appropriate for preventive services.

There are advantages and limitations to both the shotgun and rifle approaches. The shotgun approach has the appealing goal of addressing a mounting public health problem by targeting the majority of children in the population rather than a select, albeit high-risk, subsample. Despite this ambitious goal, the disappointing outcomes from community intervention trials with adults and school-based programs with children are quite sobering and do reveal a poor track record. Such data suggest that modification to previous shotgun efforts may be necessary if such approaches are to have even a modest impact on pediatric obesity.

As noted previously, however, previous shotgun approaches have primarily relied on psycho-educational information dissemination and community activism to promote weight change. More aggressive forms of environmental manipulation, including legislative changes, appear not to have been attempted on a population level. For example, changes of this magnitude might include raising taxes on high-fat breakfast foods to discourage their consumption. The success of the shotgun approach to preventing obesity might rest on the implementation of broader environmental changes than have been attempted previously.

Regarding the rifle approach, there is clearly room for more prevention studies in this area. However, it should be noted that all interventions using this approach share the common challenge of identifying the most appropriate children or adolescents for intervention. This challenge is not simple, given that not all obese children become obese adults, particularly if they are obese at younger ages *(121–123)*. Therefore, researchers run the risk of intervening with young children who might otherwise "outgrow" their obesity if left untreated, although the probability of this decreases as children remain obese into the teenage years. Future advances in the molecular genetics of obesity may help to better identify those children who are the best candidates for prevention services.

6.2. Philosophy of Prevention Methods: Inoculation vs Environmental Manipulations

Prevention strategies can be fundamentally geared toward changing the child ("inoculation") or changing the environment ("environmental manipulation"). Inoculation approaches emphasize teaching children skills to avoid overeating, to select low-fat foods, and to devote more time to physical exercise despite an increasingly sedentary environment. This philosophy has guided most school-based interventions.

On the other hand, environmental manipulations include prevention strategies, such as taxing fattening foods *(124)*, installing signs in public settings to promote activity over sedentary behaviors *(125)*, prompting in supermarkets to guide food selection *(126)*, changing the marketing of certain food products to subgroups *(127)*, developing and implementing fat substitutes and other advances in food science *(128)*, involving parents in intervention to better control their children's environment *(98)*, and directly providing nutritionally sound meals to obese individuals in lieu of them shopping for themselves *(129)*.

There appears to be no controlled studies comparing prevention programs differing on relative efficacy of inoculation versus environmental manipulations for promoting weight loss. However, the disappointing results from community prevention trials with adults and the limited success of school-based programs suggest that inoculation methods might have limited effectiveness for reducing pediatric obesity. Furthermore, as previously mentioned, environmental influences on body weight are transient *(130)*. More pervasive and sustained inoculation strategies might be necessary before effects on obesity status can be detected. As noted later, there is currently active interest in governmental policy changes that would target lifestyle modifications that could impact obesity. These controversial issues will be central to obesity prevention efforts in the near future.

6.3. Punitiveness of Prevention Efforts: Punitive vs Nonpunitive Approaches

Prevention programs can include punitive or nonpunitive strategies. The taxation of fattening foods is an example of a punitive strategy that has already been used to target alcohol and cigarette sales *(131,132)*. Examples of nonpunitive strategies include subsidizing gymnasium membership fees, price reductions for low- or fat-free foods in restaurants and supermarkets, and, perhaps, tax rebates for weight loss. Preliminary data from French and colleagues *(133)* indicated that reducing the price of fruits and vegetables in school cafeterias promoted sales of those targeted items. Similar strategies have been used in school and worksite vending machines to promote the sale of low-fat snack foods *(134)*.

There are no data comparing the relative efficacy of punitive versus nonpunitive approaches. Given this absence of data, nonpunitive strategies may be more desirable than punitive strategies strictly regarding humanitarian grounds.

6.4. Timing of Prevention Efforts: Rifle Approach

Interventions targeting high-risk children must decide the optimal time to begin intervention. One position is that there may be critical periods during the life span that are pivotal for the development of obesity and might be ideal for prevention services. For example, Dietz *(135)* suggested three possible critical periods: the first and second trimesters of pregnancy, the period of "adiposity rebound" between ages 5 and 7, and adolescence.

It has also been suggested that the earliest possible interventions might be the most efficacious *(115)*. Unlike interventions with older children, efforts targeting younger children may not need to challenge eating patterns that have been practiced over many years. Early interventions that provide overexposure to lower fat and lower total calories, rather than to higher fat and higher calorie foods, possibly promote a greater liking for these foods *(136–138)*.

On the other hand, data to support the critical periods hypothesis of obesity are mixed. For example, Allison and colleagues *(139)* concluded that the intra-uterine period was a critical period for the development of adulthood height but not BMI. Other genetics studies have also demonstrated that environmental influences on BMI are transient in duration

Table 2
Directions for Future Obesity Prevention Research Identified by the NIH Pilot Studies Investigators

Expand the types of interventions to be tested to prevent obesity and promote weight control and long-term maintenance of weight loss.	• Explore approaches such as participatory action research that encourage community input and have an influence on intervention design and content. • Develop and test interventions that target multiple societal levels, individual-based interventions, and their combination, to determine whether public health and individual approaches are interactive. • Develop and test environmental strategies (in homes, organizations, work sites, neighborhoods), including policy changes. • Develop and test family-based interventions and interventions in the primary health care setting, including physician incentives.
Conduct research to improve efficacy of interventions for obesity prevention and long-term weight control programs.	• Determine optimal intervention duration, frequency, and mode of delivery (group or individual face-to-face contact, telephone, mail, internet). • Use highly controlled study designs to determine optimal physical activity and dietary prescriptions in adults and in children and adolescents. • Determine the optimal use of meal replacements for weight loss and its long-term maintenance. • Test interactive effects of genetic and/or psychosocial predictors of obesity by stratifying study participants on these factors. • Explore and describe approaches to intervention by internet, alone and in combination with other intervention strategies. Gather information on the "dose" of intervention the internet provides and the ability of the internet to enhance motivation and adherence to interventions.
Conduct observational studies to guide interventions for obesity prevention, weight control, and long-term maintenance of weight loss.	• Identify physiological and behavioral characteristics that put individuals at risk for weight gain. • Identify physiological and psychosocial characteristics and other predictors of success in intervention programs. • Identify barriers to enrolling and participating in weight control programs. • Identify approaches and strategies to enhance motivation and adherence to intervention in children and adolescents, women during the prenatal and postpartum period, and other populations.
Use theoretical models to design obesity prevention interventions.	• Incorporate theoretical perspectives from other areas of research in the conceptualization and design of obesity prevention studies (e.g., family systems theory, and organizational behavior theory). • Conduct studies in which obesity prevention objectives are integrated with other types of health promotion or behavior change objectives.

(Continued)

Table 2 *(continued)*

• Develop more systematic concepts of how interventions can be tailored to specific behaviors, populations, and contexts. • Conduct research to improve measurement methodology for obesity preventionresearch. • Develop valid and affordable energy balance measurements suitable for large-scale trials outside of clinical settings. • Identify psychosocial measures most relevant for obesity research and explore whether these psychosocial measures can be standardized and used in weight control trials. • Determine a health-related and functional definition obesity in children.

From Kumanyika and Obarzanek (ref. *7*).

(130). Such findings are inconsistent with the critical periods hypothesis and suggest that the duration of the intervention may be more important than the age at which it is initiated.

7. FUTURE DIRECTIONS

As noted earlier, the NIH "Pathways to Prevention" Workshop convened in 2001 *(7)* to brainstorm on innovative approaches to obesity prevention research. A summary of these recommendations is provided in Table 2. Specific recommendations focused on conceptually expanded models of intervention, including those targeting societal and policy level interventions. Workshop participants addressed the importance of differentiating obesity treatment from prevention, noting that established paradigms for treatment may not necessarily translate into effective prevention strategies. Figure 1B presents a conceptual framework for obesity prevention that overlaps with other health promotion domains, including substance abuse, human immunodeficiency virus/acquired immune deficiency syndrome, and cigarette smoking. The utility of this paradigm and the efficacy of the NIH-funded pilot prevention studies will be evaluated in upcoming years.

The role of government health policy, as well as that of the food industry, in obesity prevention will likely remain prominent areas of attention in the foreseeable future. Popular books, including *Food Fight (140)* and *Food Policy (141)*, have drawn public attention to these issues. Brownell reviewed evidence for the so-called "toxic environment" and what he perceived to be a range of potential strategies for combating the problem on societal levels. Examples include government regulation of advertisements to children, taxation of select energy-dense snack foods to generate funds for health promotion campaigns, and the removal of soft drink vending machines from schools. The potential impact of these recommendations vis-à-vis obesity prevention needs to be studied empirically.

On the other hand, it appears that an increasing number of parent and lay groups may not wait for the results of controlled studies before getting actively involved. A case in point is the debate surrounding the sale of soft drinks in schools. Prospective epidemiological studies linked increased carbonated beverage consumption with obesity onset in children *(142)*, and a policy statement from the American Academy of Pediatrics recommended that pediatricians work with school administrators to decrease availability of low-nutrient energy-dense foods in schools *(7)*. Therefore, as of the writing of this chapter, certain New York City and Philadelphia school districts had banned the sale of soda in vending machines and cafeterias as a result of the advocacy efforts of parents

Table 3
Recommendations for the Prevention of Pediatric Overweight and Obesity American Academy of Pediatrics Policy Statement 2003

Health Supervision
Identify and track patients at risk by virtue of family history, birth weight, or socioeconomic, ethnic, cultural, or environmental factors.
Calculate and plot BMI once a year in all children and adolescents.
Use change in BMI to identify rate of excessive weight gain relative to linear growth.
Encourage, support, and protect breastfeeding.
Encourage parents and caregivers to promote healthy eating patterns by offering nutritious snacks, such as vegetables and fruits, low-fat dairy foods, and whole grains; encouraging children's autonomy in self-regulation of food intake and setting appropriate limits on choices; and modeling healthy food choices.
Routinely promote physical activity, including unstructured play at home, in school, in child care settings, and throughout the community.
Recommend limitation of television and video time to a maximum of 2 h/d.
Recognize and monitor changes in obesity-associated risk factors for adult chronic disease, such as hypertension, dyslipidemia, hyperinsulinemia, impaired glucose tolerance, and symptoms of obstructive sleep apnea syndrome.
Advocacy
Help parents, teachers, coaches, and others who influence youth to discuss health habits, not body habitus, as part of their efforts to control excess weight and obesity.
Enlist policy makers from local, state, and national organizations and schools to support a healthful lifestyle for all children, including proper diet and adequate opportunity for regular physical activity.
Encourage organizations that are responsible for health care and health care financing to provide coverage for effective obesity prevention and treatment strategies.
Encourage public and private sources to direct funding toward research into effective strategies to prevent excess weight and obesity and to maximize limited family and community resources to achieve healthful outcomes for youth.
Support and advocate for social marketing intended to promote healthful food choices and increased physical activity.

American Academy of Pediatrics Policy Statement: Prevention of Pediatric Overweight and Obesity. (From ref. *144*.)

and other community leaders. It is important that controlled studies test the effects of such environmental changes on children's weight change and obesity status.

Finally, the intersection of the legal and health systems, including the effects of litigation, will be an interesting issue for the future trends. For example, recent years have shown an increase in the number of lawsuits brought against certain food franchises. Perhaps the most widely publicized lawsuit was *Pelman vs McDonalds*, in which the plaintiffs claimed that a group of children became overweight and developed medical complications as a result of eating at the franchise. This controversial lawsuit was eventually dismissed from

court *(143)*; however, the case suggested that some individuals may use litigation to leverage social changes before research documents the effects of such efforts.

8. SUMMARY AND RECOMMENDATIONS

Prevention of childhood obesity is a national priority in the United States as well as in other countries worldwide where prevalence levels have changed dramatically in recent decades. The disorder is complicated because it is clearly genetically influenced, although its manifestation may depend on environmental pressures. Few studies have tested obesity prevention programs for children, although intriguing school-based data implicate the protective effects of reduced television viewing. Beyond these data, the field remains at its infancy. Pressure from lay advocacy groups is mounting for changes, at least within school settings. Regarding clinical recommendations for pediatricians, parents, and other health care providers, we conclude by providing the American Academy of Pediatrics' *(144)* Policy Statement for the Prevention of Pediatric Overweight and Obesity (*see* Table 3).

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14 Can Childhood Obesity Be Prevented?

Preschool Nutrition and Obesity

Christine L. Williams

KEY POINTS

- In the past three decades the prevalence of obesity among preschool children has doubled.
- Obesity in children 3 yr of age and older is an important predictor of adult obesity.
- Obesity in preschool children is associated with significant comorbidities as in older children and adolescents, especially dyslipidemia and elevated blood pressure.
- The USDA Food Guide Pyramid for Preschool Children provides a useful general overview of a nutrient-balanced and energy-adequate diet for 2- to 6-yr-old.
- Energy balance in preschool children is of critical importance in the primary prevention of obesity.

1. INTRODUCTION

Early childhood is a time of rapid growth, development, and learning. These characteristics are critical in defining the nutritional needs of preschool children and provide the framework for dietary guidelines and nutrition policy. Early childhood also is a time when children begin to develop eating habits that may differentially promote or discourage the development of chronic disease later in life. Therefore, the goal of preschool nutrition programs is to promote dietary habits that achieve optimal health, growth, and development in a manner that also minimizes risk of nutrition-related disease. This chapter reviews the primary preschool nutrition programs in the United States and explores the impact of recent trends in early childhood obesity, preschool dietary intake, and physical activity issues related to dietary guidelines for healthy 2- to 5-yr-old children.

2. EARLY CHILDHOOD OBESITY TRENDS: UNITED STATES

During the last two decades, the proportion of US children who are significantly overweight (body mass index [BMI] ≥ 95th percentile) has more than doubled, prompting in-depth reviews of trends in dietary intake and physical activity in childhood. Because the obesity epidemic has also extended downward to preschool children, the importance of energy balance in preschool children becomes of critical importance in the primary prevention of this disease.

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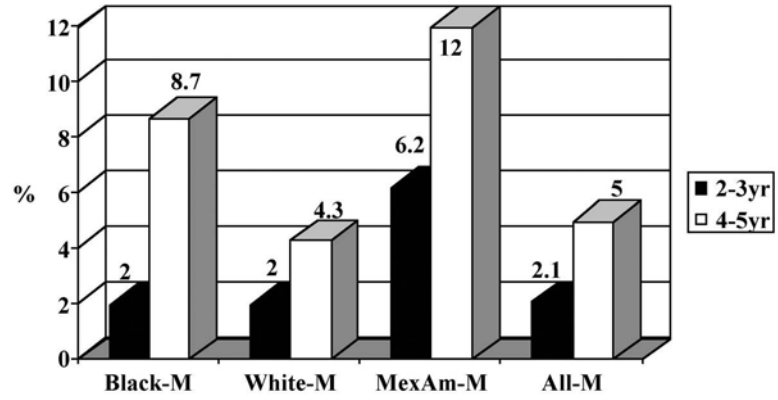

Fig. 1. Percentage of 2- to 3- and 4- to 5-yr old boys at or above the 95th percentile of the weight-for-length growth. (From Third National Health and Nutrition Examination Surveys [1988–1994].)

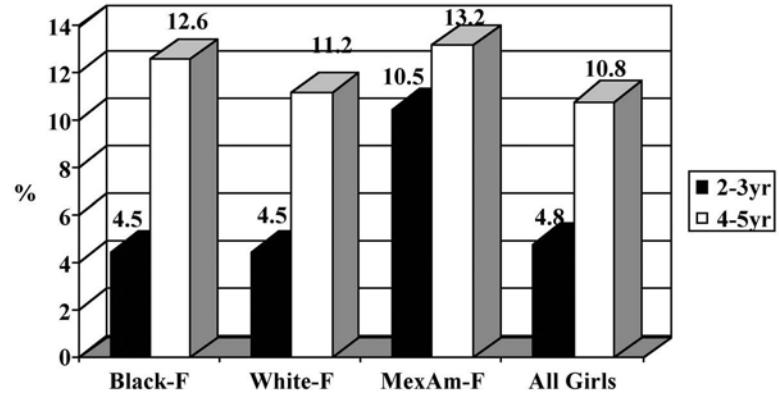

Fig. 2. Percentage of 2- to 3- and 4- to 5-yr old girls at or above the 95th percentile of the weight-for-length growth. (From Third National Health and Nutrition Examination Surveys [1988–1994].)

Excessive weight and obesity are independent risk factors for increased morbidity and mortality throughout the life cycle. For example, excessive weight and obesity in women are predictors of gestational diabetes during pregnancy and newborns with excessive birth weight *(1)*. High birth weight is a predictor of excessive weight and obesity in adulthood and of cofactors associated with insulin resistance (IR) *(2)*. In parallel with the worldwide increases in obesity prevalence, the prevalence of excessive weight and obesity in children is rising *(3)*. Because obesity in childhood frequently tracks into adulthood, increases in childhood excessive weight and obesity clearly are major contributors to the adult obesity epidemic *(4)*. Children express the same comorbidities that are associated with being overweight and obese as adults *(4,5)*. Therefore, being overweight during childhood brings with it comorbidities that will increase the duration of comorbidities in an individual by one to two decades, a factor that can increase the impact of a number of risk factors on adult diseases.

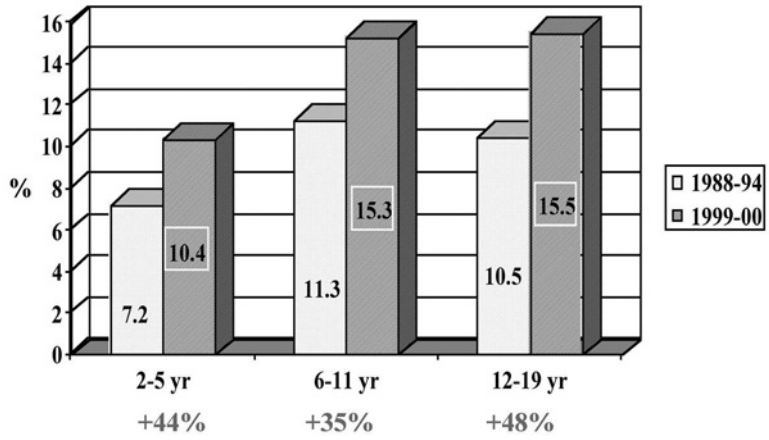

Fig. 3. Prevalence of excessive weight in US children, NHANES: 1988 to 1994 and 1999 to 2000.

The increase in the prevalence of preschool obesity has been observed in the United States and internationally. These increases have been noted in all racial and ethnic groups, but some groups have been affected more than others. In the United States, the National Health and Nutrition Examination Surveys (NHANES) databases provide alarming statistics showing substantial increases in the prevalence of excessive weight (defined as the 85th to 95th percentiles of the weight-for-height growth references) *(5)*. Presently in the United States, about 10% of children ages 4 to 5 yr are overweight (Figs. 1 and 2). The increase in prevalence across the US pediatric population generally has been similar in girls and boys. Among preschool children, girls were affected more than boys between the NHANES I and the NHANES III (1988–1994) surveys (a period of almost 20 yr); excessive weight and obesity in 4- to 5-yr-old girls doubled but increased only approx 25% among boys who were the same age. However, by the NHANES in 1999 to 2000, preschool boys and girls were similarly affected (about 10% for both genders). Among older children (6–19 yr), there has been an approximate doubling of obesity prevalence in boys as well as in girls in the United States in the past 30 yr *(5)* (Fig. 3).

Although the increases in childhood obesity have been noted in all racial and ethnic groups, some groups appear to be at greater risk of developing this disease. Excessive weight tends to be highest among Mexican-American and African-American children and lowest among non-Hispanic Caucasian children. Overall, nearly 22% of preschool children in the United States in 1999 to 2000 were classified as "at risk of overweight" (BMI: 85th to 95th percentile), and 10% were classified as "overweight" (BMI: ≥95th percentile). This compares with 18.6 and 8.5%, respectively, in 1983.

In the Bogalusa Heart Study in Louisiana, the prevalence of excessive weight among 5- to 24-yr-olds from the biracial community increased twofold between 1973 and 1994. A fact that is particularly concerning is that the yearly increases and relative weight and obesity during the latter part of the study (1983–1994) were 50% greater than those between 1973 and 1982 *(6)*. Additionally, independent of racial/ethnic differences, lower socioeconomic status (SES) is another important predictor for the high prevalence of excessive weight and obesity in US children *(5)*.

Other industrialized settings are recording similar disturbing trends in increasing obesity prevalence. In Japan, the frequency of obese school children between ages 6 and 14 yr increased from 5 to 10% and that of extremely obese children from 1 to 2% between 1974 and 1993 *(3)*. Childhood obesity is not limited to the industrialized countries. In a recent review, De Onis and Blossner *(7)* reported the rapidly increasing prevalence of excessive weight and obesity among preschool children in developing countries. Interestingly, certain countries simultaneously demonstrated high percentages of excessive weight and high frequencies of wasted (malnourished) children. Specific examples include Northern Africa, where the percentage of overweight children and wasted children exceeded 8 and greater than 7%, respectively. Similarly, in Eastern Asia, 4.3% of preschoolchildren were overweight, and 3.4% were underweight. In South America, where malnutrition and underweight were once predominant, the percentage of overweight preschool children was close to 5%, but wasted children comprised only 1.8%. In several countries (e.g., Egypt, Argentina, Malawi, Nigeria, Uzbekistan, Peru, Qatar, South America, Jamaica), the percentage of overweight children exceeds that of the United States. In 38 countries where secular data are available, 16 showed a rising trend in obesity prevalence over time, 14 were static, and only 8 showed falling rates in obesity prevalence. Rates of increase seem most marked in countries in Northern Africa, such as Morocco and Egypt, as well as in some countries of the Caribbean and South America *(7)*. Therefore, obesity in children can no longer be classified as a Western problem alone; it is now shared by nearly all industrialized areas and many developing countries. The economic burden of these trends is also significant *(8)*.

2.1. Tracking of Childhood Obesity Into Later Life

Data from a number of studies provide strong evidence that higher levels of BMI during childhood can predict excessive weight later in life. This was recently summarized in a review by Goran *(9)*. Data from four longitudinal studies were reviewed and showed that the probability of excessive weight at age 35 yr for children with BMI in the 85th to 95th percentiles increased with increasing age. The prediction for adult weight was most accurate for BMI at age 18 yr, with accuracy decreasing for BMI below age 13 yr. Goran *(9)* concluded that the "persistence of pediatric obesity into adulthood increases according to the age at which obesity is initially present." Similarly to what has been recorded in North America, obesity during childhood in Japan is associated with increased likelihood of obesity during adulthood. In a Japanese study, approximately one-third of obese children grew up to become obese adults *(10)*. Whitaker et al. *(11)* found that the risk of adult obesity was greater in both obese and nonobese children if at least one parent was overweight. This effect was most pronounced in children that were younger than age 10 yr; after age 10 yr, the child's own overweight/obesity status was a better predictor than having an obese parent. These studies show the importance of the family environment in contributing to the increasing prevalence of obesity. These increases are most likely associated with changes (increases) in food supply and caloric intake accompanied by diminishing levels of physical activity. One might consider that these family studies provide strong evidence for the genetic contribution to obesity. However, it is very unlikely that changing gene pools can explain the doubling or even tripling of obesity prevalence rates in certain groups over 20 yr, which is too short a period to affect the genetic background in affected populations.

Must et al. *(12)* presented data relating to the outcomes of overweight adolescents followed for up to 50 yr. Both men and women who were overweight at adolescence

had increased age-related morbidity and mortality relating to cardiovascular and other chronic diseases. Increased risk was also present even if adolescents who were obese had lost the excess weight during the adult period *(12)*, suggesting that obesity during adolescence may set triggers that are associated with adverse risk in the adult.

One emerging area of research involves the potential role of intra-uterine growth and growth in the first year of life for predicting the emergence of increased cardiovascular risk and obesity during adulthood. These potential links were critically reviewed by Dietz and Gortmaker *(4)*. In reviewing the Dutch Famine Studies, they noted that individuals who were exposed *in utero* to famine in the first trimester of pregnancy were more likely to be overweight at age 18 yr compared with those exposed to famine at other periods during pregnancy. In contrast, individuals exposed to famine late in pregnancy tended to be underweight at age 18 yr. Although low birth weight and low weight gain in the first year of life may contribute to increased risk of hypertension, dyslipidemia, and cardiovascular risk in the adult population, Dietz and Gortmaker concluded that it is unlikely that low birth weight contributes significantly to obesity prevalence in the adult population. Additionally, although an individual who is born overweight (>4000 g) does have a higher risk of being an overweight adult, these authors suggested that less than 5% of adult obesity is caused by individuals born with high birth weights.

2.2. Health Effects of Childhood Obesity

Obesity-associated chronic disease risk factors are present in adults and also manifest in overweight and obese children *(13,14)*. For example, data from the Bogalusa Heart Study showed that approx 60% of overweight 5- to 10-yr-old children had one cardiovascular risk factor, such as high blood pressure, hyperlipidemia, or elevated insulin levels *(15)*. From the same cohort of 5- to 10-yr-olds, greater than 20% of overweight children had two or more cardiovascular risk factors, which would substantially increase the risk of these individuals for earlier cardiovascular disease (CVD) if they were tracked into adulthood. Similarly to adults, children who were moderately overweight showed that an elevation of low-density lipoprotein cholesterol (LDL-C) levels and hypercholesterolemia did not substantially increase with higher degrees of obesity. With more marked degrees of obesity, rises in plasma triglyceride levels and decreases of high-density lipoprotein cholesterol are more common, and, similarly to adults, blood pressure elevations in children are more common with significant obesity than with moderately excessive weight. With the rising prevalence of excessive weight and obesity in children, non-insulin-dependent diabetes mellitus (type 2 diabetes) increasingly becomes a pediatrician's problem. In one report *(16)*, 4% of new diagnoses of diabetes before 1992 were classified as type 2 diabetes. In 1994, 16% of new diabetics were classified with type 2 diabetes—a fourfold increase. In the Cincinnati area between 1982 and 1994, there was a 10-fold increase in type 2 diabetes in children, and the African-American population was more severely affected than the Caucasian population *(16)*.

Alarming findings are now emerging from Asian countries that are rapidly Westernizing their lifestyle habits. For example, in urban Japanese children, plasma total cholesterol levels and LDL-C now exceed those found in US children *(17)*. A recent analysis examining differences between the Japanese and US pediatric populations suggests that dietary habits, exercise, and adiposity differences do not explain the varying lipid levels between Japanese and US children. The hypothesis was raised that the populations not previously exposed to Western diets and lifestyles may demonstrate more adverse effects on

expression of cardiovascular risk factors than longer exposed populations *(17)*. In Japanese children, excessive weight and obesity are also associated with substantial elevations of plasma total cholesterol and LDL-C levels and type 2 diabetes, and in some areas of Japan, type 2 diabetes is now more common in children than type 1 diabetes *(18)*.

Other comorbidities are also associated with childhood obesity. These include orthopedic problems, such as Blount's disease, skin fungal infections and acanthosis nigricans, hepatic steatosis and steato-hepatitis; sleep apnea; pseudotumor cerebri; and psychological and behavioral problems. Psychological problems associated with childhood obesity include negative self-esteem, withdrawal from interaction with peers, depression, anxiety, and the feeling of chronic rejection *(5)*. Therefore, a strong body of evidence suggests that BMI in childhood is associated with various adverse biochemical, physiological, and psychological effects, many of which have the possibility of tracking into chronic disease risk factors in adulthood.

There are different biological effects related to excessive weight and obesity among different racial/ethnic groups. Evidence is available to suggest that aerobic capacity may be lower in African-American than in Caucasian children and may be more significant than energy expenditure leading to obesity *(9)*. Goran *(9)* concluded that fasting insulin and acute insulin response are significantly higher and insulin sensitivity is significantly lower in African-American than in Caucasian prepubertal children; these differences are not explained by differences in body fat, body fat distribution, diet, or physical activity. These findings are important because they suggest that prevention and treatment strategies may require different approaches in different racial/ethnic populations.

Although hereditability estimates of genetic population studies suggest that 40 to 70% of adult obesity results from genetic factors, there is little information available in pediatric populations. Nevertheless, statistics show that a proportionate variance within adult populations is accounted for by genetic factors; however, this does not reflect the interplay between genes and environment at the individual level, especially within the developing individual.

Finally, many of the childhood obesity comorbidities result in significant economic cost to society. This is apparent in the proportion of pediatric hospital discharges with obesity-related diagnoses, a figure that has increased dramatically in the past 20 yr. Wang and Dietz *(8)* analyzed the economic burden of obesity in youths ages 6 to 17 yr and found that obesity-related annual hospital costs (based on 2002 constant dollar value) increased more than threefold over the two decades between 1979 to 1981 and 1997 to 1999: from $35 million to $127 million. Discharges of obesity-related diabetes nearly doubled (1.43 to 2.36%); obesity-related gallbladder disease tripled (0.36 to 1.06%); and obesity-related sleep apnea increased fivefold (0.14 to 0.75%). Asthma and certain mental disorders were the most common primary diagnoses when obesity was listed as the secondary diagnosis. Thus, the increasing prevalence and severity of obesity among children and adolescents has resulted in significant economic costs as well.

2.3. Prevention of Early Childhood Obesity

In approaching early childhood obesity, it is helpful to consider three levels of prevention: primordial prevention, aimed at maintaining a normal BMI; primary prevention, aimed at preventing "at risk of overweight" children (BMI: 85th to 95th percentiles) from becoming overweight (BMI: ≥95th percentile); and secondary prevention, aimed at treating obese children (BMI: ≥95th percentile) to reduce comorbidities and reduce (or possibly normalize) the severity of excessive weight *(5,14)*.

2.3.1. Primordial Prevention

The most desirable prevention goal would be the primordial prevention of obesity, that is, preventing children with desirable BMI (<85th percentile) from becoming "at risk of overweight " (BMI: 85th to 95th percentile). Success in achieving this goal is most likely to occur if prevention strategies and interventions are initiated in the first few years of life, perhaps even beginning during the prenatal period and infancy.

There are several reasons for emphasizing primordial prevention, the most significant of which are related to adipocyte physiology, adiposity rebound, and the limited potential for reversing metabolic changes associated with obesity. During fetal life, babies begin to develop fat cells (adipocytes) at around 15 wk of gestation *(19)*. Rapid increase in fat cell number and size, especially during the third trimester of pregnancy, results in an increase in percent body fat from about 5 to 15% *(20)*. Thus, by full-term birth, about one-sixth of the infant's weight is fat (a 6-lb baby would have about 1 lb of fat). This translates into about 5 billion adipocytes—16% of the 30 billion found in adults.

During infancy, fat cells increase primarily in size rather than number. Between ages 2 and 14, there is little change in fat cell *size* in lean children, and there is only a small increase in fat cell number from between ages 2 and 10 yr *(21–23)*. Among obese children, however, fat cells continue to increase in size *(24)*. Eventually, this triggers an increase in fat cell number when adipocyte lipid content exceeds about 1 μg of lipid per cell *(25,26)*. During therapeutic weight loss, the rate at which new fat cells are formed (from pericapillary fibroblasts) slows in obese children but still continues at a rate that is greater than in lean children *(27)*. Therefore, once obesity has developed in childhood, it may be restrained, but perhaps not reversed, giving added impetus for primordial prevention.

2.3.2. Infant Weight Gain, Adiposity, and Obesity

Numerous studies have demonstrated an association between caloric intake and adiposity in infancy. Dewey and Lonnerdal *(28)* found that caloric intake was related to weight-for-length at age 2 mo but not at age 4 or 6 mo. Average energy intake over the first 6 mo of life correlated significantly with weight gain and weight-for-length at 6 mo. Others reported that British infants consumed significantly more calories and demonstrated twice the prevalence of excessive weight than comparable Swedish infants *(29)*. For 6- to 9-mo-old infants, no correlation was found between adiposity and caloric intake when using 3-d food intake records *(30)*.

However, rate of weight gain during the first year of life is a powerful predictor of adiposity at age 7 yr in both boys and girls. Weight gain above the 90th percentile during infancy was associated with a relative risk of obesity of 3.3 and 1.7 for boys for girls, respectively, at age 7 yr *(31)*.

Some studies have suggested that mode of infant feeding (i.e., breast vs bottle, early vs later introduction of solids) is an important determinant of childhood obesity. Evidence is mixed, however, because studies are confounded by problems, including the fact that many infants get mixed feedings of both bottle and breast; bottle-fed babies often consume solids earlier, and lack of control for SES, maternal age, and race. Many studies suggest that bottle-fed infants tend to consume more calories and gain weight more rapidly than breast-fed infants *(32)*. In a study that controlled for SES, maternal age, and race, Kramer et al. *(33)* found that breastfeeding conferred a significant long-term protective effect against obesity in childhood. Breastfeeding combined with delayed introduction of solid foods exerted a protective effect against obesity to age 2 yr *(34)*. Although other studies have not found any differences in development of adiposity

between bottle- and breast-fed infants *(35)*, advocating breastfeeding has other benefits and can be recommended for these reasons in addition to the possible benefit in preventing accelerated weight gain.

2.3.3. Feeding and Activity Patterns of Obese and Lean Infants and Toddlers

Several investigators have explored differences in feeding and activity patterns of lean and obese infants to determine the earliest times at which clear-cut differences can be identified and also to determine which factors might be etiologically more important in the initiation of childhood obesity. Roberts et al. *(36)* measured both energy intake and expenditure at ages 6, 9, and 12 mo among infants of normal-weight and overweight mothers. At age 6 mo, infants of overweight mothers consumed 42% more calories than infants of normal-weight mothers. There was no difference at ages 9 and 12 mo. At age 3 mo infants of obese mothers who were becoming overweight already had a total energy expenditure (TEE) that was an average of 20.7% lower than all normal infants combined. Griffiths et al. *(37,38)* studied energy intake and expenditure in preschool children and also found that the children of obese parents had a significantly lower TEE (22% lower) compared with children of normal-weight parents. However, In this study, self-reported energy intake was also lower. Collectively, these data suggest that perhaps both early overfeeding plus reduced TEE contribute to infant obesity or that infants and children who are susceptible to obesity have a variety of mechanisms for providing the surplus energy for excessive weight gain, including excess intake of energy as well as low TEE in physical activity.

Other investigators have reported evidence that soon after birth, the feeding behaviors of heavier infants differ from that of leaner infants. Compared with lighter infants, heavier infants suck faster and consume more formula if the formula is sweetened. Babies with medium or increased adiposity showed increased sucking rates and pressures (greater avidity of feeding) for sweet-tasting fluids compared with thinner babies, who demonstrated a relative aversion for sweet taste. This behavior predicted relative weight at age 3 yr. Overall, sucking studies found that infants who suck more frequently at higher pressures and with shorter intervals between bursts of sucking consume more calories and tend to be fatter. These characteristics explained about 25% of the variance in adiposity (triceps skinfold thickness) at age 2 yr in a multiple regression analysis. The strongest positive correlation was between sucking pressure and triceps skinfold thickness at age 2 yr. Interestingly, number of feeds per day was negatively correlated with adiposity, with fewer feeds associated with greater triceps skinfold thickness *(39)*.

Tracking studies indicate that the obese infant has an increased risk of becoming an obese older child, although overall only one-fourth to one-third tend to show persistence of the problem, and the most significant predictor is parental obesity *(31,40–42)*. Generally, the longer that the obesity persists and the more severe the problem, the greater the likelihood it will persist into adulthood. Parental obesity increases the likelihood that all of these factors will occur.

2.3.4. Adiposity Rebound

The importance of primordial prevention is also supported by studies of adiposity rebound in childhood. Normally, BMI increases from birth to age 1 yr, then falls and reaches a low point at around age 6 yr before rebounding again. In several studies to date, the timing of adiposity rebound in childhood was related to risk of adolescent and adult obesity. Rolland-Cachera et al. *(43)* studied the pattern of BMI changes during childhood

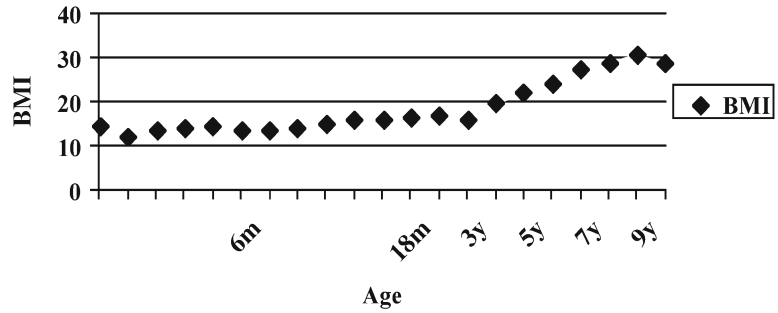

Fig. 4. Adiposity rebound before age 3 yr. Case History: White female, BW 8 lb, 7 oz; 20.5 inches; Mom-Ht 66, Wt 225 lb; Dad-Ht 73, Wt 210 lb; TC = 189, HDL = 29, LDL = 99; Trig 309; TC-skinfold 22 mm (age 6); BP 94/64 (approx 90%); Ht 90 to 95%; Wt > 95%. Favorite foods: pizza, cheeseburgers, chicken-nuggets, hotdogs, cookies, pudding, cheese.

for 151 children ages 1 mo to 16 yr. They reported that early adiposity rebound before age 5.5 yr (vs later adiposity rebound) was associated with significantly greater adiposity at age 16 yr *(43)*. They noted that infants who eventually became obese by age 14 yr were slightly heavier at birth, reached a higher peak BMI slightly later in infancy (14 mo vs 12 mo), showed less of a decline in BMI at about age 2 yr, and began adiposity rebound at a mean age of 4 yr (50 mo) versus 6 yr (70 mo) for lean children/teens. Figure 4 illustrates changes in BMI for an overweight 6-yr-old child in whom adiposity rebound occurred at age 3 yr.

Whitaker et al. *(42)* conducted a retrospective cohort study using lifelong height and weight measures recorded in outpatient medical records. He found that a younger age at "adiposity rebound" was associated with an increased risk of adult obesity and that the risk was independent of BMI at adiposity rebound and independent of parent obesity *(42)*. Therefore, the earlier the age at which adiposity rebound occurs, the more likely a child will become an obese adult. Thus, one of the most compelling arguments for initiating obesity prevention in the first few years of life is the desire to delay adiposity rebound.

Although adiposity rebound was first described 15 yr ago, little is known about *what* triggers early adiposity rebound. It is likely to be influenced by a complex interaction of both genetic and environmental factors. In view of the increasing prevalence of early adiposity rebound among children and adolescents, the mean age of adiposity rebound might also be decreasing in the United States. To date, however, no data have reported temporal trends for adiposity rebound in the United States or other developed countries. Additional studies are needed to determine if dietary factors (e.g., high fat) promote early adiposity rebound or if inactivity also plays a role.

There are some clues that accelerated weight gain in preschool children may be associated with an increasing proportion of calories from fat in the diet. This might be expected because fat is very energy-dense relative to protein and carbohydrates, thus foods high in fat typically are higher in total energy and smaller in volume (an important satiety cue). High-fat foods also are very palatable (which encourages overeating) but not highly satiating; therefore, children may be less likely to adjust subsequent dietary intake to compensate for having consumed a high-fat (high-calorie) meal. Dietary fat is stored more efficiently than dietary protein or carbohydrates, and, similarly to carbohydrate and protein, oxidation of dietary fat does not increase as dietary fat intake increases.

Klesges et al. *(44)* evaluated determinants of accelerated weight gain over 3 yr in a cohort of 146 preschool children. Higher baseline levels of %energy (%E) from fat were

associated with greater increases in adiposity and BMI, as were recent increases in %E from fat. Total caloric intake did relate to weight gain; however, when fat kcal were entered into the regression equation, it explained more of the variance in weight gain than total energy intake. This suggests that for preschool children, the percentage of calories from total fat is an important contributor to accelerated weight gain. Baseline aerobic activity and increased leisure activity were also significant predictors of change in BMI. These modifiable diet/activity variables together accounted for 33% more of the variance in body mass change than the combined set of nonmodifiable variables (such as parental obesity). In the same study, Eck et al. *(45)* found that percent of calories from fat, but not total calories, were higher in a high-risk group of 3- to 4-yr-old children who gained more weight over a 1-yr period of time.

Robertson et al. *(46)* also found that total energy intake was higher among preschool children who had accelerated weight gain over a 2-yr period compared with children whose weight gain for height was normal. Children with accelerated weight gain also consumed a greater proportion of their calories from fat *(46)*. Daheeger et al. *(47)* reported that number of calories from dietary fat was a significant predictor of fat mass for 4- to 7-yr-old boys (even after controlling for total caloric intake) but not for girls.

Also, obese preschool children often are prompted by their parents to eat more at mealtimes. Klesges et al. *(48)* sent trained meal observers into homes to observe families at mealtimes and found that obese children were prompted to eat more, offered more food, presented with more food, and encouraged to eat more food twice as often or more than lean children.

A major rationale for very early primordial and primary prevention is that obesity in children ages 3 yr and older is an important predictor of adult obesity. Whitaker et al. *(42)* found that this was true regardless of whether the parents were obese. Additionally, parental obesity more than doubled the risk of adult obesity among both obese and nonobese children less than age 10 yr. In contrast, obese children younger than age 3 yr whose parents were not obese were not at increased risk of adult obesity. The fact that parental obesity is a powerful predictor of child obesity has been known for several decades. With the currently increasing rates of adult obesity, however, the prevalence of parental obesity increases, as does the risk that children of these obese parents will also become obese.

2.3.5. Secondary Prevention

Secondary prevention is also important, because both duration and severity of obesity are critical predictors of persistent childhood and adolescent obesity and eventual adult obesity and related health problems (i.e., the longer obesity persists during childhood and adolescence, and the more severe it becomes, the greater the likelihood that the problem will persist into adult life). Thus, there is good rationale for believing that obesity prevention efforts should be initiated during the preschool period for maximum effectiveness.

2.3.6. Balancing Energy Input With Energy Expenditure

Because obesity represents the net effect of an imbalance between energy intake (diet) and energy expenditure (physical activity) over time, there is a critical need to help susceptible children balance energy intake with energy output. This is often difficult, considering the present lifestyle of children and families in the United States. For example, in 45 min of exercise, a 75-lb child may be expected to expend approx 90, 135, 180, and 525 calories during continuous bicycling, walking, dancing, and running, respectively. In contrast, a regular size "fast food" meal would provide about 600 calories, but a "supersized fast food" double cheeseburger meal could provide more than

Table 1
Goals for Obesity Prevention Initiatives in Childhood

Perinatal: Supply good prenatal nutrition and health care; avoid excessive maternal weight increase; control diabetes; help mothers lose weight postpartum; and offer nutrition education.

Infancy: Encourage breastfeeding and continue breastfeeding for 6 mo or longer, if possible; provide a balanced diet; avoid an excess of high-calorie desserts and snacks; and follow weight increase closely.

Preschool: Provide early experiences with foods and flavors; help develop healthy food preferences; encourage appropriate parental feeding practices; monitor rate of weight increases to prevent early adiposity rebound; and provide child and parent nutrition education.

Childhood: Monitor weight increase for height (slow down if excessive); avoid excessive prepubertal adiposity; provide nutrition education in school; provide healthy food choices in the school food service (fruits, vegetables, whole grains, low saturated-fat content); provide daily physical activity opportunities in gym, recess, and after-school programs for an hour of moderately vigorous active play.

Adolescence: Prevent excess weight increase after growth spurt; provide nutrition education in high school; provide healthy food choices in the cafeteria (fruits, vegetables, whole grains, low saturated-fat content); provide daily physical activity opportunities in gym and sports program, during and after school.

1800 calories. Therefore, for many children who chose the larger portions (even for a child who is spends an hour a day in moderately vigorous physical activity), the result is still an excess of caloric intake over expenditure.

Strategies for preventing excess weight gain for height in childhood and preventing overt obesity emphasize plant-based foods, whole grains, fruits and vegetables, low-fat dairy products, lean meats, and less frequent "light" snacks to reduce consumption of energy-dense foods and increase consumption of nutrient-rich foods that are less energy-dense, or "lighter." However, at various ages and stages of childhood development , obesity prevention strategies differ. Table 1 summarizes suggested components of obesity prevention initiatives affecting infants and children.

For children who are significantly overweight, the goal should be to reduce severity of obesity and to treat, reduce, and eliminate comorbidities (e.g., hypertension, dyslipidemia, IR, and type 2 diabetes). For energy balance, measures are needed for children to lose weight or to slow the rate of gain and to grow into their expected heights. This requires some reduction in energy intake and substantial increases in energy expenditure *(5,14,49,50)*. In summary, therefore, childhood obesity is increasing at epidemic rates, even among preschoolchildren, and is accompanied by significant comorbidities and health problems. Prevention should be the primary goal and, if successful, will help reduce adult obesity and obesity-related chronic diseases.

3. DIETARY INTAKE IN EARLY CHILDHOOD: WHAT ARE PRESCHOOL CHILDREN EATING?

3.1. Healthy Eating Index Scores for Preschool Children in the United States

The Healthy Eating Index (HEI) developed by the US Department of Agriculture (USDA) has been used to examine the diet of all Americans, including that of preschool

Table 2
Healthy Eating Index: Overall and Component Mean Scores and Percentages for Children (Selected Years 1994 to 1996[a], 1998)

	1994 to 1996			*1998*		
Component	*Ages 2 to 3*	*Ages 4 to 6*	*Ages 7 to 9*	*Ages 2 to 3*	*Ages 4 to 6*	*Ages 7 to 9*
HEI score						
Overall	73.1	66.9	65.9	74.3	68.4	67.9
1. Grains	8.3	7.2	7.7	8.5	7.6	7.9
2. Vegetables	5.9	4.9	5.1	6.3	5.1	5.6
3. Fruits	7.1	5.4	4.4	7.4	5.8	5.0
4. Milk	7.2	7.3	7.6	7.4	7.7	7.6
5. Meat	6.3	5.3	5.5	6.6	5.6	5.9
6. Total fat	7.4	7.3	7.2	7.3	7.4	7.3
7. Saturated fat	5.4	5.6	5.6	5.4	5.7	6.2
8. Cholesterol	9.0	8.9	8.8	8.6	8.7	8.5
9. Sodium	8.8	8.1	6.9	8.7	7.5	6.1
10. Variety	7.7	7.0	7.2	8.0	7.5	7.7
Percentage of children meeting the dietary recommendations for each component						
1. Grains	54	27	30	57	31	34
2. Vegetables	30	16	20	35	19	22
3. Fruits	56	30	18	60	35	25
4. Milk	43	43	49	45	50	50
5. Meat	28	14	17	29	17	13
6. Total fat	40	37	35	39	38	38
7. Saturated fat	27	28	27	30	30	39
8. Cholesterol	84	83	81	82	81	78
9. Sodium	65	53	33	61	40	32
10. Variety	49	38	39	53	46	46

[a] The 1994 to 1996 HEI scores reflect an updated HEI methodology, therefore they are not directly comparable to previously published HEI scores.

children. This index consists of 10 components, each representing different aspects of a healthful diet *(51)*. Components 1 to 5 measure the degree to which a person's diet conforms to the USDA's Food Guide Pyramid serving recommendations for the following five major food groups: grains (bread, cereal, rice, and pasta); vegetables; fruits; milk (milk, yogurt, and cheese); and meat/meat alternatives (meat, poultry, fish, dry beans, eggs, and nuts). Component 6 measures total fat consumption as a percentage of total food energy (calorie) intake. Component 7 measures saturated fat consumption as a percentage of total food energy intake. Components 8 and 9 measure total cholesterol intake and total sodium intake, respectively. Component 10 measures the degree of variety in a person's diet. Each component of the HEI has a maximum score of 10 and a minimum score of 0. Intermediate scores are computed proportionately. High component scores indicate intakes close to recommended ranges or amounts. The maximum combined score for the 10 components is 100. An HEI score greater than 80 implies a good diet, an HEI score between 51 and 80 implies a diet that needs improvement, and an HEI score less than 51 implies a poor diet.

In data collected between 1994 and 1998 as part of the USDA Continuing Survey of Food Intake in Individuals, only about 25% of children ages 2 to 5 yr consumed a "good diet" (HEI score > 80) (Table 2). The majority, about two-thirds, consumed diets ranked

Table 3
Diet Quality: Percentage of Children Ages 2 to 9 Yr by Age and Diet Quality (as Measured by the Healthy Eating Index [HEI], Selected Years 1994 to 1996[a], 1998)

Characteristic	*Ages 2 to 5*	*Ages 6 to 9*
1994 to 1996		
At or below poverty		
Good diet	18	10[b]
Needs improvement	71	81
Poor diet	11	9[b]
Above poverty		
Good diet	26	14
Needs improvement	67	78
Poor diet	7	8
1998		
At or below poverty		
Good diet	22	18[b]
Needs improvement	70	74
Poor diet	8	8[b]
Above poverty		
Good diet	29	12
Needs improvement	66	80
Poor diet	5	8[b]

[a] The 1994 to 1996 HEI scores reflect an updated HEI methodology, therefore they are not directly comparable to previously published HEI scores.

[b] Sample size relatively small to make reliable comparisons.

Note that an HEI score above 80 implies a good diet, an HEI score between 51 and 80 implies a diet that needs improvement, and an HEI score less than 51 implies a poor diet.

(From the US Department of Agriculture, Center for Nutrition Policy and Promotion, Continuing Survey of Food Intakes by Individuals.)

as "needs improvement," with HEI scores between 51 and 80. "Poor" diets were consumed by 7 to 10% of preschoolers (Table 3). For preschool children in families at or below poverty, only 22% consumed a "good diet" compared with 29% of preschoolers in families with income above poverty level *(51)*.

3.2. Child and Adult Care Food Program Menus

Recently, the USDA compared the macro- and micronutrient content of menus of young children who participate in the Child and Adult Care Food Program (CACFP) to nutrient standards *(52)*. The standards used in the USDA study included the "Dietary Guidelines for Americans recommendations *(53)* as the standard for percent of energy from total fat and saturated fat. Recommended Daily (Dietary) Allowances (RDAs) *(54)* provided the desirable levels for calcium, vitamins A and C, iron, and energy. The Diet and Health Report of the National Research Council *(55)* supplied recommendations for carbohydrates, sodium, and cholesterol. Analysis of these menus revealed that children are being fed meals that are too high in fat. Although all menus that were examined in the USDA study generally followed the established meal-pattern guidelines for child nutrition programs *(56,57)*, the menus for breakfast served in CACFP programs supplied more than one-fourth of the recommended intake for all key nutrients, and although goals were met for total fat, cholesterol, carbohydrates, and sodium, they were

not met for saturated fat and energy. Lunches provided more than one-third of the recommended intake of vitamins A and C and calcium, and although the goal for cholesterol was met, the goals for total fat, saturated fat, iron, sodium, carbohydrates, and energy were not. Snacks provided 10% or more of the recommended intake for key nutrients and energy. A contemporaneous study conducted in Mississippi provided findings that were consistent with the findings in USDA report *(58)*. An analysis of CACFP menus from these preschool daycare programs indicated that the total fat and saturated fat content of their lunches exceeded levels recommended in the Dietary Guidelines for Americans *(53)*.

3.3. National Surveys of Diet Intake Among Preschool Children

3.3.1. What Are US Children Eating and How Does This Compare With Current Guidelines?

There have been a number of national surveys of dietary intake in the past several decades, all of which included data on children. Although there are methodological differences that should be taken into consideration when analyzing secular trends in dietary intake, these surveys remain the best source of data available at the present time *(59–70)* National surveys of dietary intake include the NHANES I, II, III, and 1999–2000; the Nationwide Food Consumption Surveys (NFCS) of 1977–1978 and 1987–1988; and the USDA Continuing Surveys of Food Intake of Individuals (CSFIIs).

A review of trends in dietary fat intake in children over age 2 yr reveals that: (a) children's intake of dietary fat, saturated fat, and cholesterol tends to reflect the trend for the population as a whole; (b) the percent of daily calories from dietary fat (%kcal) has decreased over the past decade, although the total intake of fat (grams per day) may not have decreased because caloric intake also may have increased; (c) a significant proportion of children still consume amounts of total and saturated fat that are above currently recommended levels; and (d) although there are some differences in fat intake by ethnicity and poverty levels, these differences tend to be small *(68)*.

The nutrient intake of childrens ages 3 to 5 yr who were surveyed in the 1995 CSFII *(70)* and the 1987–1988 NFCS *(71)* is summarized in Table 4. Total energy intake ranged between 1390 and 1576 kcal/d, protein intake was 14 to 15% of daily energy, and carbohydrate intake accounted for about 50 to 55% of energy intake. Saturated fat intake (expressed as %E intake) was 12 to 13%E in both surveys, and total fat intake was 32 to 35%E, with the lower value reported in the more recent 1995 CSFII . Mean dietary cholesterol intake was 182 to 201 mg/d, below the usual 300 mg/d goal but above the more stringent 100 mg/1000 kCal goal. Mean dietary fiber intake was 8.9 to 11.2 g per day, meeting the minimal goal of "Age + 5" g per day for this age group *(72)* but falling short of the more optimal "Age + 10" g per d *(72)* or the National Academy of Sciences fiber intake goals of 19 and 25 g/d for 1- to 3-yr and 4- to 8-yr-old children *(73)*.

The proportion of US preschool children who met dietary guidelines for total fat, saturated fat, and dietary cholesterol intake was reported by geographic areas *(74)*. This data was compared with dietary intake data collected between 1994 and 1996 for 3- to 5-yr-old low-income preschool children in nine Head Start Centers in upstate New York. Between 31 and 37% of US preschoolers consumed the recommended 30% or less of kcal from total fat, with more children in the northeast meeting the goal than in

Table 4
Nutrient Intake of Preschool Children in Selected US Dietary Surveys

Age 3 to 5 yr	*CSFII, 1995*	*NFCS, 1987 to 1988*
Energy, kCal/d	1576	1390
Protein, %E	15	14
Carbohydrate, %E	50	55
Total fat, %E	32	35
Saturated fat, %E	12	13
Cholesterol, mg/d	182	201
Dietary fiber, g/d	11.2	8.9
Iron, mg/d	12.9	10.1
Zinc, mg/d	9.1	7.5
Calcium, mg/d	829	781
Vitamin A, µg RE/d	842	na
Vitamin C, mg/d	102	78
Vitamin E, mg/d	5.3	5.3

(From refs. *46* and *47*.)
Healthy Start, 258 intervention, 213 control children.

Table 5
Percent of Preschool Children (Age 3–5 yr) Who Meet Dietary Guidelines for Total Fat, Saturated Fat, and Cholesterol

	Healthy Start 24-h intake at baseline in 3- to 5-yr-olds	*NHANES-III (1994–1996): 1-d dietary intake for 3- to 5-yr-old children by geographic region in the United States*			
% KCal	*New York*	*Northeast*	*South*	*Midwest*	*West*
Total fat at or less than 30%E	46.3	37.2	31.6	31.5	31.2
Saturated fat below 10%E	30.4	23.6	23.3	23.5	21.4
Cholesterol at or less than 300mg/d	88.3	89.0	86.2	90.7	84.9

Healthy Start Preschool Children (1995–1997) compared with NHANES III data on 3- to 5-yr-old preschool children in the United States (1994–1996).
Healthy Start, $n = 471$ children in nine Head Start centers (New York).

other parts of the United States. Fewer children (21–24%) met the goal for saturated fat intake, consuming less than 10% of kcal from this source. A much higher proportion of children met the goal for dietary cholesterol intake, with 85 to 90% consuming less than 300 mg per day. Among the low-income, predominately minority, preschool children in the New York Head Start Centers *(75)*, a greater proportion were within dietary goals for total (46%) and saturated (30%) fat. However, the majority of children still exceeded recommended intakes of both (Table 5).

In a sample of 30 4- to 6-yr-old children, Goran et al. *(76)* measured TEE by doubly labeled water technique. TEE averaged 1379 calories per day. Fonteville et al. *(77)* found a TEE of 1383 kcal/d for 5- to 6-yr-old children, and Davies et al. *(78)* found a TEE of 1172

kcal/d for 1- to 4-yr-old children. Thus, the energy intake values obtained in the CSFII and NFCS are consistent with measured TEE, despite the fact that the RDA for energy for 4- to 6-yr-old children is 1800 kcal/d—a 12 to 23% overestimate of energy requirements.

4. DIETARY GUIDELINES FOR PRESCHOOL CHILDREN

4.1. The USDA Preschool Food Guide Pyramid

The USDA Food Guide Pyramid for Preschool Children provides a useful general overview of a nutrient-balanced and energy-adequate diet for 2- to 6-yr-old children. This guide is helpful to child health professionals and parents because it highlights the variety of food groups that should be included in the diet of preschool children and also describes portion sizes. The key elements of the Preschool Food Guide Pyramid are provided in Fig. 5.

4.2. Specific Recommendations for Nutrient Intake in Preschool Children

Recommendations for dietary intake in early childhood account for the child's unique growth and developmental patterns, specific nutrient requirements, vulnerability of sentinel nutrients, factors important to the establishment of healthy eating habits, and nutritional risk factors for childhood- and adult-onset diseases. The role of dietary fat as a concentrated source of calories as well as essential fatty acids (EFAs) must be balanced with a desire to avoid excessive fat intake, which is associated with increased risk of future chronic disease.

All US national dietary guidelines include the 2- to 5-yr-old preschool group. Little controversy exists regarding recommended intake of most vitamins, minerals, and protein in childhood. More of the controversy exists regarding recommended intakes of total fat and types of fat in the diet as well as regarding intakes of total carbohydrate and its constituents (particularly simple sugars and starch). Many health organizations have developed dietary guidelines for children at least partly based on the role of dietary fat (especially saturated fat) in the early development of atherosclerosis *(79–83)*. This rationale is strengthened by studies linking a high intake of dietary fat with some human cancers *(84–86)* and obesity *(87–89)*.

Current US dietary guidelines for children set forth by the American Academy of Pediatrics and others recommend no more than 30%E from total fat, less than 10%E from saturated fat, and no more than 300 mg of cholesterol daily *(68,79,80)*. These guidelines do not apply to infants and toddlers younger than age 2 yr, because there is a consensus that a higher fat intake is needed to support the rapid growth and caloric requirements of this age group.

These guidelines were first proposed by the 1985 National Consensus Development Panel, cosponsored by the National Institutes of Health (NIH) and the American Heart Association (AHA) *(83)*. They recommended numerical guidelines for dietary fat intake for all Americans younger than age 2 yr, with total fat intake at no more than 30% of calories and saturated fat at less than 10% of calories. In 1991, these guidelines were endorsed by the National cholesterol Education Program (NCEP) Report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents *(80)*. The American Academy of Pediatrics, which in 1983 and 1986 had declined to recommend numeric fat intake guidelines for children, was represented on the NCEP panel, and in 1992 also recommended "an average daily intake of 30% of total calories from fat, <10% from saturated fat, and <300 mg of cholesterol per day."

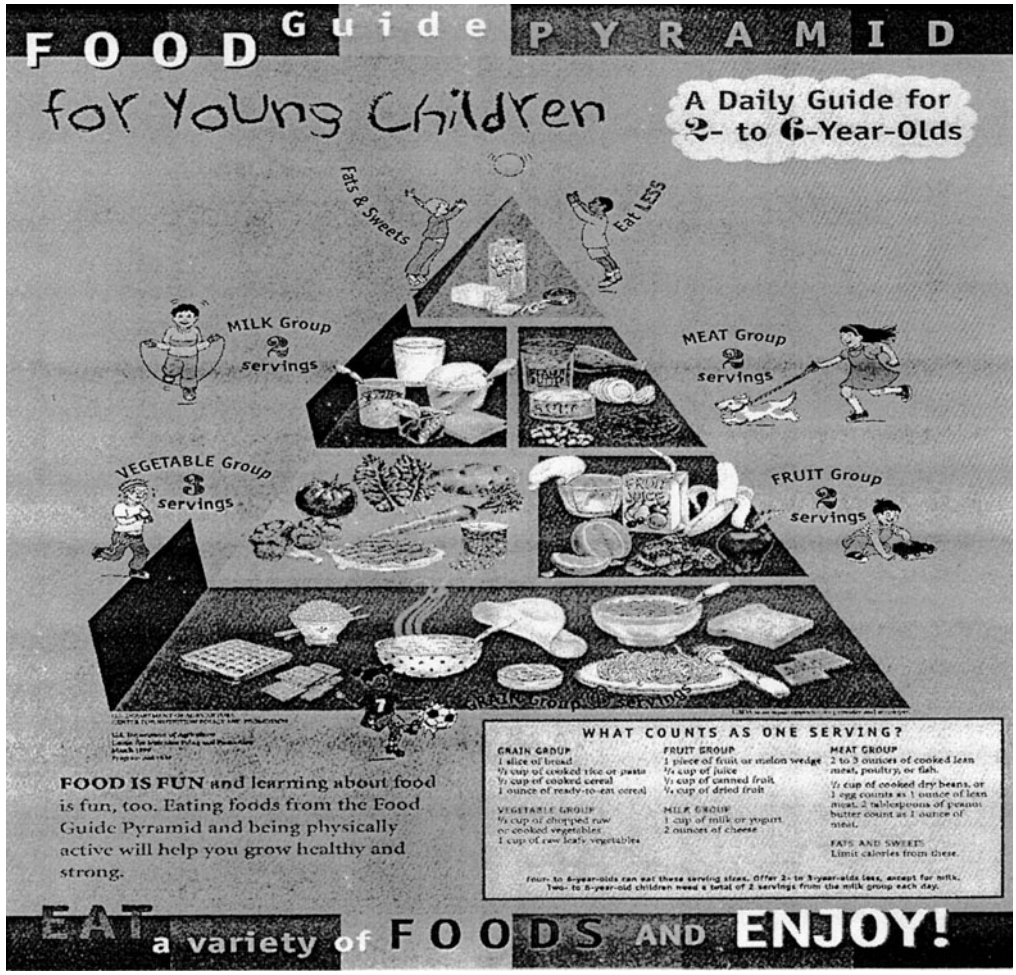

Fig. 5. The USDA Preschool Pyramid.

The National Health Promotion and Disease Prevention Objectives (Healthy People 2010) also recommended that Americans reduce dietary fat intake to an average of 30%E or less and average saturated fat intake to less than 10%E among people ages 2 yr and older *(82)*. Therefore, at present, the American Academy of Pediatrics, the AHA, the NIH, the USDA, the Food and Drug Administration, and at least 26 other health-related organizations recommend numerical limits for dietary fat intake for Americans older than age 2 yr *(80)*.

The age at which to initiate the fat-controlled or prudent diet varies somewhat according to the source of the recommendation. The AHA recommends that the prudent diet be adopted after age 2 yr. NCEP considers age 2 to 3 yr as "transitional" between the high-fat diet of infancy and the fat-modified prudent diet. It suggests that "toddlers 2 and 3 years of age may safely make the transition to the recommended eating pattern as they begin to eat with the family" *(81)*. The USDA Dietary Guidelines suggest that preschool children should gradually reduce dietary fat over a longer period of time, reaching the 30%E from total fat goal at about age 5 yr *(68)*.

There may be some rationale for establishing a preschool dietary transition period to avoid an abrupt drop in fat intake after age 2 yr and a possible abrupt drop in calories. However, there is sufficient research to show that when intake of calories and essential nutrients are adequate, dietary fat intake can be safely reduced to 30% of kcal after age 2 yr without compromising growth and development *(90–96)*.

4.3. Rationale for Fat/Saturated Fat Guidelines in the Preschool Period

Recommendations to reduce dietary fat beginning after age 2 yr stem from scientific consensus on the role of dietary fat (especially saturated fat) in the early development of atherosclerosis. The NCEP panel explained that "a variety of studies indicate that the process of atherosclerosis begins in childhood; that this process is related to elevated levels of blood cholesterol; and that these levels are often predictive of elevated blood cholesterol in adults" *(97–102)*. Autopsy studies have demonstrated that early coronary atherosclerosis or precursors of atherosclerosis often begin in childhood and adolescence. In the aorta, fatty streaks begin to develop soon after birth and are almost universal by age 3 yr. Fatty streaks in the coronary arteries lag behind by about a decade. Studies such as the Pathological Determinants of Artherosclerosis in Youth (PDAY) Autopsy Study have suggested that the critical stage of the disease process on which we need to focus prevention strategies is during the conversion of childhood fatty streaks into thicker raised lesions *(102)*. More research also needs to clarify how the conversion occurs and which risk factors influence it. Although atherosclerosis may not be totally preventable, evidence suggests that its progress can be substantially retarded, thus deferring the resulting heart attacks to much later in life.

Dietary fat (and especially saturated fat) is associated with high levels of blood cholesterol, a major independent risk factor for atherosclerosis. Elevated cholesterol levels are highly prevalent in children—even in toddlers over age 2 yr *(103–107)*. In a survey of 3- to 5-yr-old children in New York, 26% had levels in the borderline-high range (170–199 mg/dL), and 11% were in the high range (<200 mg/dL) *(108)*. National survey data on cholesterol levels in children are available from NHANES II (1976–1980) and, more recently, from NHANES III (1988–1994). Currently, about 10% of adolescents ages 12 to 17 yr have total cholesterol levels exceeding 200 mg/dL (considered "high"); additionally, about 30% have borderline-high levels of 170 to 199 mg/dL. Regional surveys provide additional evidence that a higher proportion of children are currently in the elevated risk categories than were there previously *(109,110)*.

Plasma levels of total cholesterol and LDL-C show significant tracking from childhood to adulthood *(111,112)*. For example, the Bogalusa Heart Study found that about 70% of children in the top quintile for blood cholesterol could be found in the top two quintiles 12 yr later *(111)*. The proportion was similar for children originally on the bottom quintile (70% could later be found in the bottom two quintiles for cholesterol) *(111)*. Obesity strengthens the tracking of both elevated cholesterol and elevated blood pressure, a factor of some importance as the pediatric population becomes more obese *(112)*.

Numerical guidelines for fat intake in childhood are also based on considerations of nutritional adequacy. When the dietary intake of children is examined regarding sources of excess calories, 15% are derived from fat and 10% are derived from sugar. These calories replace the consumption of fruits, vegetables, and grains, which would result in meeting RDAs for macro- and micronutrients in the diet. Thus, a moderate reduction in

fat intake in childhood, if replaced by healthy fruits, vegetables, and grains, would improve the overall quality of children's diets *(113)*.

4.4. Early Diet Behavior and Diet Behavior in Later Childhood

There is some evidence that dietary patterns established in childhood persist into later life. Children develop food preferences through their early eating experiences, and these may be sustained over time. The majority of studies examining dietary patterns over time have considered tracking of nutrient intake in cohorts of children. Data on such tracking is available from the Bogalusa Heart Study as well as the Framingham Children's Study *(114,115)*.

In the Bogalusa Heart Study, there were a number of significant correlations for 12-yr tracking of macronutrient intake in the range of 0.2 to 0.4. These were significant despite the fact that they were based on a single 24-h dietary recall at each data collection point. More recent data from the same group report somewhat higher correlation coefficients, in the range of 0.4 to 0.5 *(114)*. Similarly, the Framingham Children's Study reported on tracking of nutrient intake in a preschool population. They reported that 40 to 57% of children in the highest quintiles for total and saturated fat intake were still in this quintile 2 to 3 yr later *(115)*. Less is known regarding actual food consumption over time—that is, do young children who eat large amounts of fruits, vegetables, milk, cereal, or sweets continue to do so later in adolescence and adult life?

4.5. Dietary Fat and Obesity in Childhood

There is some evidence to suggest that a high-fat diet and sedentary lifestyle are the two key features that have created the current epidemic of obesity in the United States and other affluent countries. In physiological studies, fat plays a powerful role in undermining human body weight regulatory systems. This is partly because the body has the ability to increase the rate at which it oxidizes carbohydrate and protein when ingestion of these macronutrients is increased. The body must do so because it has a very limited ability to store these nutrients. However, when large amounts of dietary fat are consumed, the body is unable to increase rates of oxidation to re-establish fat balance. Additionally, oxidation of fat is suppressed when an excess energy or any of the other macronutrients is consumed. Therefore, the human metabolism is not designed to cope with excess fat consumption *(87–89)*.

Dietary fat is also not particularly good at satisfying hunger (i.e., it has low satiating power). This is especially true for obese individuals. Manipulation of the fat content of diets results in reproducible spontaneous fat loss during low-fat diets and fat gain during high-fat diets. Individuals consuming a high-fat diet develop "passive overconsumption," because it occurs unintentionally and without consuming excess bulk. Additionally, some obese individuals have a behavior preference for high-fat foods *(87,89)*.

Obesity in US children has been increasing over the past several decades *(116–118)*. Although the percentage of calories consumed as fat has declined, the total amount of calories consumed appears to have increased *(64,65,68)*. Therefore, the amount of fat consumed each day in grams may not have changed. Additionally, relatively low levels of physical activity are the norm. Daily physical education in the schools has declined—particularly in high school and especially among older girls *(119,120)*. In 1996, 23% of children in grades 4 to 12 had no physical education *(120)*. Therefore,

based on metabolic considerations, physical activity needs to increase and dietary fat needs to decrease to achieve the synergism needed to prevent childhood obesity.

It is known that the earlier the age at which adiposity rebound occurs, the more likely a child will become an obese adult *(121)*. Whitaker et al. *(11)* conducted a retrospective cohort study using lifelong height and weight measures recorded in outpatient medical records and found that a younger age at "adiposity rebound" was associated with an increased risk of adult obesity and that the risk was independent of BMI at adiposity rebound and of parent obesity. Thus, adult obesity occurs more frequently in children who have early adiposity rebound. Additional studies are needed to determine if the age of adiposity rebound among US children has become younger over the past several decades and also if a high-fat diet promotes early adiposity rebound.

4.6. Heart-Healthy Preschool Meals

Promoting heart-healthy behaviors during childhood has become the focus of school-based programs aimed at the primary prevention of atherosclerosis and coronary heart disease (CHD) *(122–127)*. The majority of school-based programs have targeted elementary school children and include dietary intervention studies targeting both food service and individual dietary behavior change. Generally, these studies demonstrate that the total and saturated fat content of school meals can be improved without compromising physical growth or micronutrient intake.

In the Healthy Start preschool project *(75)*, the food service intervention was successful in decreasing the saturated fat content of preschool menus from 12.5 to 8.95%E in the intervention schools (28.4% decrease), whereas control schools increased saturated fat content by 6.6% (Table 6). This intervention was modeled after methods developed by the Child and Adolescent Trial for Cardiovascular Health (CATCH; ref. *122*), which also was able to decrease the saturated fat content of school lunch menus from 14.8 to 12.0% E in intervention schools (18.9% decrease), whereas control schools decreased only 9.3%. For total fat, the Healthy Start preschool intervention decreased the total fat content of school lunch menus from 30.8 to 25.6%E in intervention schools (16.9% decrease), whereas control schools increased by 1.4%. The CATCH intervention decreased the total fat content of school lunch menus from 38.7 to 31.9%E in intervention schools (17.6% decrease), whereas control schools decreased only 6.9%. Both Healthy Start and CATCH succeeded in decreasing total and saturated fat in school lunches yet maintained adequate intake of energy and other essential nutrients *(75,128)*.

Although numerous strategies were used in the Healthy Start study to reduce the saturated fat content of preschool meals and snacks, reducing the fat content of milk and other dairy products used in the preschool centers was commonly employed. This is consistent with the findings of Sigman-Grant and others *(129,130)* who have suggested that, although a number of strategies could be effective in lowering the saturated fat content of children's diets, use of only skim milk as a minimum strategy could be effective in achieving saturated fat goals in young children yet maintaining adequate intake of other essential macro- and micronutrients. Implementing a few key strategies, such as switching from whole or 2% fat milk to 1% fat milk, draining and rinsing cooked ground meat, and removing skin and all other visible forms of fat from poultry and meats, all contribute to the overall reduction of saturated fat content in school meals and dietary intake by young children.

Table 6
Changes in Total and Saturated Fat Content of School Menus at Baseline and After a Food Service Intervention: The Healthy Start Preschool Project and the CATCH Elementary School Project

	Intervention		*Control*		
% KCal	*Baseline*	*Follow-up*	*Baseline*	*Follow-up*	
Preschool/school menus					
Total fat	30.8	25.6	29.5	29.9	Healthy Start
	38.7	31.9	38.9	36.2	CATCH
Saturated fat	12.5	8.95	12.1	12.9	Healthy Start
	14.8	12.0	15.1	13.7	CATCH
24-h intake					
Total fat	30.6	30.0	30.4	30.3	Healthy Start
	32.7	30.3	32.6	32.2	CATCH
Saturatedfat	11.8	11.4	12.4	12.0	Healthy Start
	12.7	11.4	12.5	12.1	CATCH
School meal intake					
Total fat	26.8	27.2	25	29.4	Healthy Start
	*	*	*	*	CATCH
Saturated fat	9.9	9.4	10.4	11.8	Healthy Start
	*	*	*	*	CATCH

*Not available.

The Healthy Start study demonstrated that it is possible to decrease the saturated fat content of children's diets in the preschool population without compromising energy intake or overall nutrient adequacy of the diet. All nutrient values at baseline and follow-up, with the exception of zinc, met or exceeded one-third of the RDAs and/or Dietary Reference Intakes for the 1- to 3- and 4- to 6- or 4- to 8-yr-old age group. Zinc values in the menus as well as in dietary intake were low relative to the other nutrient values attained in the menus and through diet, possibly because of the prominence of milk (a rich source of calcium but not of zinc) in the menus. Therefore, initiatives like Healthy Start demonstrate that targeted food service interventions in preschool centers can be a safe and effective way of increasing the proportion of children who consume heart-healthy, lower saturated fat meals and snacks consistent with current dietary guidelines *(75)*. For children, this is one part of an integrated chronic disease prevention agenda aimed at the primary prevention of atherosclerosis and CHD, obesity, type II diabetes, hypertension, and other chronic diseases linked to dietary patterns of overnutrition.

4.7. Safety Issues Related to a Fat-Controlled Diet in Early Childhood

In the past, concern has been raised that fat-controlled diets in early childhood might fail to provide adequate energy for normal growth and development. Dietary fat is a concentrated source of calories, and calories are essential for growth. Studies over the past decade have sought to determine the relative importance of caloric versus fat intake

in supporting normal growth and development in childhood. Results of the most recent growth and safety studies indicate that growth is adequately supported in diets in which fat contributes approx 25 to 30% of calories if there is adequate intake of calories, high quality protein, and essential nutrients *(90–96)*.

Reports from the Special Turku Coronary Risk Factor Intervention Project (STRIP) study in Finland are particularly relevant to the safety issue. Their data indicate that a diet low in saturated fat and cholesterol, even when started in infancy, can be compatible with normal growth and development as long as caloric and essential nutrient intakes are adequate *(90,131,132)*. The STRIP Baby Project is one of the few studies in which dietary fat reduction was initiated even before age 2 yr. This is an ongoing prospective randomized trial in 1062 Finnish infants randomly assigned at age 8 mo to usual diet (control) or to a diet with reduced total fat, saturated fat, and cholesterol. Results show that absolute energy intakeas well as parental BMI and height best predict child growth patterns and that children with consistently low fat intake grow as well as children with higher fat intake. Additionally, intakes of vitamins, minerals, and trace elements did not differ between the intervention and control groups *(131)*. In a recent report, neurological development was similar in intervention and control groups at age 5 yr, providing further evidence of the safety of the "heart-healthy" diet in early childhood *(132)*.

Theoretical studies of computer modeling of nutrient adequacy provide specific levels of dietary fat. These studies indicate that the fat-modified diet need not be deficient in calories or any essential nutrients. However, care must be taken to ensure adequate intake of some nutrients, such as calcium, iron, and zinc *(68)*. This is supported by the findings of both observational and intervention studies in children. Some of these studies involve preschool-age children, whereas others involve older, prepubertal children or adolescents.

The Studies of Childhood Activity and Nutrition studies provided data regarding the nutritional status of children who consumed varying amounts of dietary fat. In a 2-yr study of 215 preschool Hispanic children, Basch et al. *(133)* found no difference in crude measurements of growth between the low-fat and high-fat groups. However, calcium levels were below recommended levels in the low-fat group, so the need to keep up intake of low-fat milk was emphasized. Wosje et al. *(135)* compared growth in toddlers who consumed either whole fat milk or 2% milk from age 12 to 24 mo. They found that despite lower intakes of total and saturated fat, total energy intake was similar in both groups, as was growth.

A diet limiting fat and cholesterol intake could result in a lack of cholesterol for myelin formation in the nervous system and for production of steroid hormones that use cholesterol as a substrate *(134)*. However, cholesterol is not an essential nutrient. In healthy children, the body is capable of synthesizing cholesterol for all of it needs. There is also concern that a fat-restricted diet could lead to mineral deficiencies, especially calcium, iron, and zinc (if red meats are avoided). However, when calcium intake is sustained with adequate consumption of low-fat dairy products, both calcium and dietary fat goals can be achieved. Iron deficiency is most prevalent during the first 2 yr of life, when a low-fat diet is not recommended. Iron deficiency has been reported to be a problem among preschoolers, especially those below poverty levels. For example, 3.9% of 3- to 5-yr-old children above poverty had iron deficiency anemia, compared with 9.7% below poverty (NHANES II 1976–1980). Including lean red meat and iron-fortified products such as cereal in the diet helps prevent this deficiency. Similarly, zinc is often a shortfall nutrient for children under age 10 yr. Clinical deficiency in US children is not common; however,

it may be associated with growth retardation in Mexican-American children *(136)*. Fortified cereal products and inclusion of a variety of lean meats, complex carbohydrates, and vegetables would preclude deficiency of this mineral.

Concern exists that deficiencies in fat-soluble vitamins and EFAs may occur if dietary fat is restricted. Linoleic (18:2 n–6) and α-linolenic (18:3 n–3) acids are EFAs that cannot be synthesized *de novo* by humans and must be obtained from the diet. EFAs should provide at least 3% of the caloric intake in a normal diet. Generally, if about half of the child's fat intake comes from plant sources, the EFA requirements are met. In a 1- to 3-yr-old child's diet, 3% would be about 39 calories or 4.3 g of EFA; whereas in a 4- to 6-yr-old child's diet (which consists of 1800 kcal), 3% would be about 54 calories, or 6 g of EFA (out of the total 60 g of total fat in a 30%E fat diet). Vegetable oils are good sources of EFAs. However, it should be noted that the *trans*-isomers of linoleic acid do not have EFA activity. Deficiencies in linoleic acid are rare but include dermatitis (scaly skin), hair loss, diarrhea, and poor wound healing *(137)*.

5. FEDERAL PRESCHOOL NUTRITION PROGRAMS IN THE UNITED STATES

In the United States, two large federally funded nutrition programs are focused heavily on preschool children: the The Special Supplemental Nutrition Program for Women, Infants and Children (WIC) program (administered by the Food and Nutrition Service of the USDA) and the Head Start program (administered by the Head Start Bureau, Administration for Children and Families, Department of Health and Human Services). Both programs are linked through an interagency agreement to coordinate some services on a local, state, and federal level. Excellent overviews of these programs and summary statistics may be found on the Internet websites for the programs. Some key elements of these programs are described here.

5.1. The WIC Program

WIC is administered by the Food and Nutrition Service of the USDA. WIC was established in 1972 to counteract the negative effects of poverty and poor nutrition on prenatal and pediatric health. WIC provides a combination of direct nutritional supplementation, nutrition education and counseling, and increased access to health care and social service providers for pregnant, breastfeeding, and postpartum women, infants, and children up to age 5 yr. In the 10 yr between 1988 and 1998, the WIC program vastly expanded, with the number of enrollees growing from 3.4 million to more than 8 million; over the same period, annual WIC food expenditures grew from about $1.4 billion to $2.8 billion. Demographically, Hispanics have become a larger proportion of the caseload (Hispanics were 21% of the caseload in 1988 and 32% in 1998). Other trends over this decade showed that pregnant women and infants decreased slightly as a percent of all WIC participants; breastfeeding women more than doubled as participants; and children rose as a percentage of all WIC participants, from 47% in 1988 to 51% in 1998. Most women enrolled in WIC are between the ages of 18 and 34 yr; about half are married, and one-third have not completed high school. The average size of the WIC family or economic unit is 4.0 persons. Most WIC participants reside in single-family households, but 15% reside with extended family in multifamily households. The income cutoff for WIC eligibility is 185% of the federal poverty level. Nearly two-thirds of WIC participants

reside in families with income below the poverty level, and 23% have income between 100 and 150% of the poverty level. Although WIC families are in low-income categories, nearly three-fourths of all WIC participants reside in families with wage earners. Most WIC enrollees receive health insurance from the government's Medicaid Program (58%); about one-fourth have employer-provided insurance.

5.1.1. Nutritional Aspects of WIC Participation

Nearly one-half (47%) of families with WIC participants receive some other source of food assistance at the time that they enroll. USDA programs are the most common source of assistance, with 33% of families receiving food stamps, 27% participating in the National School Lunch Program, and 21% participating in the School Breakfast Program. Local food pantries are a source of food assistance for less than 5% of WIC families. On average, WIC families spend $25 per person on food each week, with the great majority (85%) of food spending on food at home. Fifteen percent of WIC participants report that they are "food insecure without hunger," 7% are "food insecure with hunger, moderate," and about 2% are "food insecure with hunger, severe."

By far, the most prevalent nutrition risk among all WIC enrollees is the failure to meet dietary guidelines. More than two-thirds of all women and children who enroll in WIC are at dietary risk. The risks that are most common among children, other than failure to meet dietary guidelines, are risk of anemia (24%), excessive weight (17%), and short stature (14%). Many infants do not exhibit a specific nutrition risk but are enrolled in WIC on the basis of their mother's current enrollment and documented risk; 42% of all infants have no other risk. Common nutritional risks for women are excessive weight (43%) and risk of anemia (30%).

WIC participants receive supplemental foods as one of their WIC benefits. Depending on participant category (pregnant, postpartum, or breastfeeding woman, infant, or child), WIC provides a monthly set of vouchers for nutritious foods to supplement the diet. Generally, WIC vouchers are for specific foods that are high in protein, calcium, iron, and vitamins A and C. WIC foods include iron-fortified infant formula and infant cereal, iron fortified adult cereal, vitamin C-rich fruit or vegetable juice, eggs, milk, cheese, and peanut butter or dried beans or peas. Although they do not receive WIC foods, exclusively breast-fed infants are included as WIC participants and their mothers receive an enhanced food package that includes larger quantities of WIC foods, such as tuna and carrots.

5.1.2. Excessive Weight Among WIC Children

The prevalence of excessive weight (BMI: 95th percentile or greater) among WIC children was 11% in 1992 and 13.2% percent in 1998, according to the revised age- and gender-specific NCHS growth charts (CDC, 2000); this represents a 20% increase over this 6-yr period. Prevalence of excessive weight among boys exceeded prevalence of excessive weight in girls by 1.3 percentage points in both 1992 and 1998. For boys, prevalence excessive weight increased from 11.6 to 13.9% percent over the 6-yr period (a 19.8% percent rise in prevalence); for girls, prevalence of excessive weight increased from 10.3 to 12.4% over the 6-yr period (a 22.3% percent rise in prevalence). The percent of WIC children who were overweight in 1998 tended to decrease with age, with 15.6, 14.2, 11.1, and 9.9% overweight at ages 1, 2, 3, and 4 yr, respectively.

Overweight WIC children were similar to other WIC children in that their most common nutritional risks were inadequate or inappropriate nutrient intake and low

hematocrit or hemoglobin. However, overweight children were more likely to have multiple nutrition risks compared to other WIC children: 79.1% of overweight WIC children had two or three nutrition risks, whereas only 48.2% of other children had two or three risks.

Prevalence of excessive weight among WIC children varies by racial/ethnic group, with Hispanic and Native American children having the highest rates of prevalence of excess weight (16.4 and 18.6%, respectively, in 1998). From 1992 to 1998, African-Americans had the smallest increase in prevalence of excessive weight (0.9 percentage point) and Hispanics had the largest increase (2.0 percentage points). In relative terms, however, the increased prevalence for Caucasian children, from 9.2 to 11.1%, was a 20.7% increase over the 6-yr period and was the greatest relative increase among racial/ethnic groups. Geographical differences in prevalence of excessive weight have narrowed over time. In 1992, the Northeast and Western regions had the highest rates of prevalence of excessive weight; however, these regions experienced the smallest growth in prevalence of excessive weight between 1992 and 1998. On a state-by-state basis, 10% percent of WIC children were overweight in the median state in 1992; by 1998, more than 10% of WIC children were overweight in all but five states.

5.2. Head Start

Head Start and Early Head Start are comprehensive child development programs that serve children from birth to age 5 yr, pregnant women, and their families. They are child-focused programs and have the overall goal of increasing the school readiness of young children in low-income families. Beginning with a task force recommendation in 1964 for the development of a federally sponsored preschool program to meet the needs of disadvantaged children, Head Start has grown to serve children from birth to age 5 yr as well as their families. Section 645 of the Federal Head Start Act (42 U.S.C. 9840) establishes income eligibility for participation in Head Start programs by reference to the official poverty line, adjusted annually according to changes in the Consumer Price Index. In 2002, the federal poverty level for a family of four was $18,100.

In 2002, there were 18,735 Head Start Centers in the United States, serving 905,235 children, 90% of whom were ages 3 and 4 yr. Approximately equal proportions of Hispanic (29.7%), African-American (33.8%), and Caucasian (29.9%) children were enrolled in the program, at an average cost per child of $6633. Three-fourths (77%) of Head Start families had annual incomes less than $15,000, and 60% of the children were enrolled in Medicaid.

The reauthorization of the Head Start Act in 1994 established a new Early Head Start program for low-income families with infants and toddlers. In fiscal year 2001, more than $550 million was used to support nearly 650 programs to provide Early Head Start child development and family support services in all 50 states as well as in the District of Columbia and Puerto Rico. These programs served more than 55,000 children under age 3 yr.

Head Start programs must provide opportunities for health and nutrition education to children and parents. For children, health and nutrition learning activities are integrated into the overall child development curriculum. Typically, children are involved in daily health activities such as brushing their teeth, washing their hands, and eating family

style meals. Other learning activities such as preparing foods, growing foods, reading stories, singing songs, and playacting around food themes are included throughout the program year. These health and nutrition activities are planned and carried out in a manner that promotes the individual cognitive, physical, and social-emotional development of each child.

As part of Head Start's focus on parent involvement, the program must also provide parents with the opportunity to learn the principles of preventive health, including the selection and preparation of foods to meet family needs and the management of food budgets. Parents must also have opportunities to discuss and learn about the individual nutritional status of their child and how to access services that may be needed to meet identified nutrition needs. Programs are required to provide ongoing follow-up to ensure that identified nutritional needs are met and that opportunities for nutrition education pertaining to individual nutrition concerns are available. A comprehensive preschool health curriculum developed for Head Start centers is the Healthy Start curriculum (www.Healthy-Start.com), which includes two nutrition units for classroom activities linked to specific learning objectives and also includes bilingual parental educational materials.

Head Start centers also provide children with nutritious meals and snacks. Every child in a part-day program receives meals and snacks that provide at least one-third of daily nutritional needs. Every child in a full-day program receives meals and snacks that provide one-half to two-thirds of daily nutritional needs, depending on the length of the program. All children in morning programs who have not received breakfast at the time they arrive at the Head Start Program are served a nourishing breakfast. Infants or toddlers in center-based care must receive food appropriate to their nutritional needs and developmental readiness. Meals are provided at no cost and must meet USDA Child and Adult Care Food Program meal pattern requirements. Foods served must be high in nutrients and low in fat, sugar, and salt. A variety of foods is served, which broadens each child's food experiences, and mealtime is considered a learning opportunity that is integrated into the overall curriculum. Opportunities are provided for children to be involved in food- and mealtime-related activities. Staff, children, and volunteers eat together in a family style and, to the extent possible, share the same menu.

5.3. Obesity Prevention Strategies in Preschool and Daycare Settings

5.3.1. Preschool Health Education

A potentially effective but low-cost public health approach to primordial and primary obesity prevention at the preschool age would involve dissemination and implementation of comprehensive preschool health education programs in nursery schools, daycare, and Head Start centers in the United States. In 1995, almost two-thirds of mothers with children younger than age 6 yr were working outside the home (nearly double the rate of 20 yr ago), and an increasing proportion of their children (about 3 million presently) are cared for in preschool or daycare centers. The idea of providing universal preschool for all children is gaining momentum.

Health education and interventions aimed at preschoolers are extremely important from a developmental point of view because health habits are acquired and practiced during the preschool period, health knowledge is gained, and early attitudes about health begin to be established during this period. Therefore, comprehensive health education

should be part of every preschool program as the basic minimal obesity prevention program for all children and parents.

Preschool children actually acquire a large number of health behaviors, including safety behaviors (seatbelts, helmets, hats and sunscreen, avoiding danger); dietary behaviors (food preferences and choices); physical activity behaviors (preference for active or inactive play); hygiene behaviors (washing hands, brushing teeth, etc.).

For disease prevention, it is important that health education programs are are comprehensive in nature, because such programs include health education curriculum for the children; ; teacher training; parent education; cook training; and adequate physical activity. This is in addition to legal and specific program requirements for medical and dental check-ups, immunizations, safety policies, smoking policies, medication and emergency policies, and health professional staff support.

An example of a comprehensive preschool health education program is the "Healthy Start" program (www.Healthy-Start.com) *(138)*. The Healthy Start children's curriculum was developed with a basis in social learning theory *(139)* and was designed to be developmentally appropriate for children ages 3 and 4 yr. The curriculum has 12 units, which cover a wide variety of health topics such as nutrition, physical activity, self-esteem, and drug abuse prevention, and are designed to be taught in nursery school for 20 min three times a week throughout the school year. The emphasis is on exploratory hands-on activities, games, and acquisition of new skills, knowledge, and healthy attitudes *(139)*. Additionally, there is teacher training, take-home education materials for parents, a food service modification manual, and a computer-assisted knowledge quiz for evaluation *(75,139–144)*.

The effectiveness of preschool health education programs in preventing childhood obesity would likely be significantly enhanced if they were part of a larger national public health campaign focused on reduction of obesity for the population as a whole. For children, a more global public health campaign would also include identification and early treatment of at-risk and already obese children and adolescents, which would be carried out by pediatricians and other health professionals in a variety of settings.

Obesity results from a positive net energy balance (more calories are consumed than expended), and the major modifiable factors in the equation are caloric intake (diet) and caloric expenditure (physical activity). Therefore, the primary goals of a preschool prevention strategy must be to help children, parents, and teachers achieve a fat-controlled heart-healthy diet as well as daily physical activity goals (at least 30 min a day for children over age 2 yr) *(145)*. Dietary goals include reducing the total and saturated fat in preschool meals and snacks and increasing dietary fiber with more grains, vegetables, fruits, and legumes as well as helping parents and teachers do the same at home. Physical activity goals include helping preschool centers develop adequate and developmentally appropriate physical activity programs for the children as well as helping parents and teachers initiate more personal and family physical activities for themselves and their families.

The dietary fat goals are consistent with the 1998 American Academy of Pediatrics Guidelines, which recommend that children ages 2 yr and older consume no more than 30% (and no less than 20%) of their daily energy intake (kcal/d) as fat and less than 10% of energy intake as saturated fat *(146)*. However, most preschool children still are not meeting these goals. Data from NHANES III (ph1) indicate that 77 to 85% of children and adolescents ages 2 to 19 yr exceed recommended intake of total fat, and

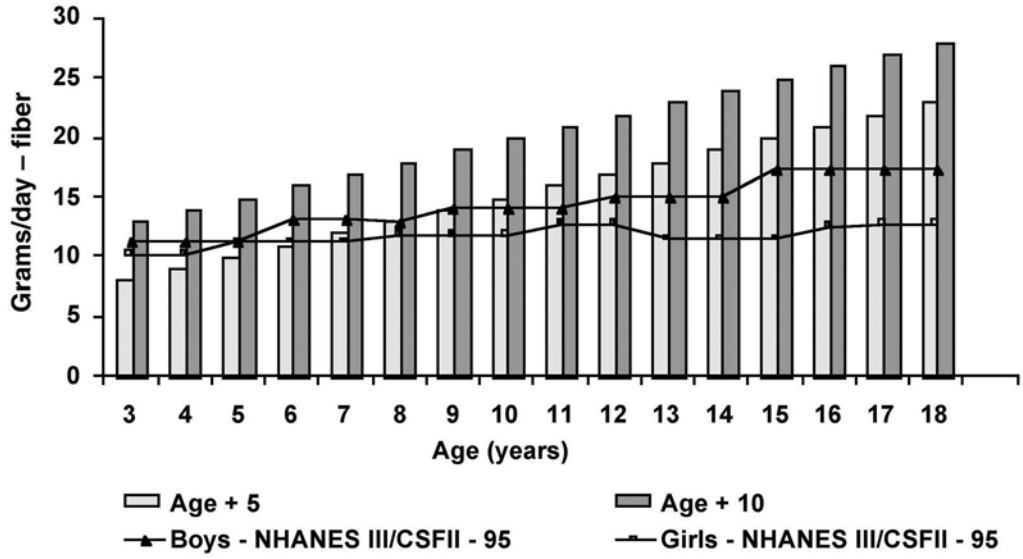

Fig. 6. Current vs recommended fiber intake for children ages 3 to 18 yr (age + 5 minimal to age + 10 maximal).

91 to 93% exceed recommended intake of saturated fat *(68)*. In the 1995 CSFII *(139)*, two-thirds of children still exceeded recommended goals (63–69% exceeded the total fat goal of < 30%E, and 62–77% exceeded the saturated fat goal of < 10%E).

Dietary fiber is also important in childhood because dietary fiber often replaces some of the fat . According to the "Age + 5" dietary fiber rule *(76,147)*, 3- to 5-yr-old children should be consuming 8 to 10 g of fiber at a *minimum* (Fig. 6). Only 54% of children in this age group meet this goal. The Bogalusa Heart Study also found that 10-yr-olds in the top quartile for dietary fiber intake also had the lowest intake of fat *(148)*.

In the "Healthy Start" project *(75)*, saturated fat in preschool meals and snacks was reduced from about 10 to 9% of energy in intervention sites (compared with an increase from 10 to 12% in control sites), resulting in lower blood cholesterol levels among preschool children. Growth rates were not affected, because calorie intake remained normal. Other studies, such as the STRIP study in Finland, have proved that growth depends on total energy intake rather than fat intake and that preschool children grow normally as long as a balanced diet with adequate energy and essential nutrients is provided *(90,149)*.

Young children also need more and better physical activity programs. If obesity is increasing among preschool children, then greater effort is needed to improve the duration and intensity of daily physical activity. In preschool centers, there is a tendency to try to make the children "sit down and be quiet!" Even at recess on the playground, children generally are left to their own devices to run around and play. Experts suggest that preschool children should be actively taught new motor skills and then helped to practice and perfect them *(150)*. This includes motor skills such as running, jumping, hopping, throwing, kicking, standing on one foot, doing a somersault, and riding a tricycle. Children who acquire these skills develop a sense of accomplishment—and then enjoy being physically active *(151)*. We also need to encourage communities to help their children be more physically active and to provide neighborhoods that are safe for children.

5.4. Obesity Prevention Strategies for Families With Young Children

5.4.1. Diet for a Healthy 2- to 6-Yr-Old Child

The Food Guide Pyramid for young children from the USDA more clearly defines the number of servings of foods from each area of the pyramid that should be consumed by young children *(152)*. More importantly, it clarifies portion size for this age group, because in the past, there has been confusion as to what constitutes a portion size for a young child. For most 2- to 6-yr-olds, energy requirements as well as macro- and micronutrient needs will be met if they consume six servings of the grain groups, three servings of vegetables, two servings of the fruit group, two servings of the milk group, and two servings of the meat group over an average of 3 d. Generally, children ages 2 to 3 yr will consume fewer calories and smaller portions (about two-thirds of a regular portion size) than older children. By age 4 yr, regular portion sizes apply. The key features of healthy "Food Pyramid eating" for preschool children can be summarized as follows:

- A good estimate of a serving for a 2- to 3-yr-old child is about one-third of what counts as a regular Food Guide Pyramid serving.
- Children ages 2 to 3 yr need the same variety of foods as older children but usually need fewer calories.
- Younger children eat smaller portions than older children. They should be offered smaller servings and allowed to ask for more. This will satisfy their hunger and not waste food.
- By the time children are age 4 yr, they can eat amounts that count as regular Food Guide Pyramid servings eaten by older family members—that is, one-half cup of fruit or vegetable, one-fourth cup of juice, one slice of bread, and 2 to 3 ounces of cooked lean meat, poultry, or fish.
- New foods should be offered in small "try me" portions (e.g., 1–2 tablespoons). Children may not begin to like a new food until they have tasted it many times and have become familiar with it.
- Eating a variety of foods every day is important for the whole family. Offer children a variety of foods from the five major food groups, and let them decide how much to eat.
- Children ages 2 to 6 yr need two servings from the milk group each day. After age 2 yr, drinking low-fat (1% or skimmed) milk will help preschoolers achieve a heart-healthy diet and avoid excessive intake of saturated fat.
- Young children's appetites can vary widely from day to day, depending on growth rates, environmental factors, and energy expenditure in play.
- As long as children have plenty of energy, are healthy, are growing well, and are eating a variety of foods, they are probably getting enough of the nutrients they need from the foods they eat.

The USDA Food Guide Pyramid does not specify a recommended fat content for milk and cheese or recommend lean versus fattier meats or grain products. Guidelines from the American Heart Association recommend a fat-modified diet after age 2 yr. The Academy of Pediatrics *(137)* recommends that after age 2 yr, preschool children should gradually adopt a fat-modified diet that provides no more than 30%E (and no less than 20%) from fat by age 5 yr. One of the easiest ways to achieve this goal is for healthy children over age 2 yr to consume low-fat dairy products, such as 1% low-fat milk *(130)*. This strategy, coupled with adequate consumption of vegetables, fruits, and fiber-containing grain products and limiting intake of added sugars and fats (the "tip" of the pyramid) will result in an energy-adequate, calorically balanced, and fat-controlled diet *(152)*. In addition to fat-controlled, healthy pyramid eating for preschoolers, daily physical activity is essential ("An hour a day of active play"). These three key heart-healthy

Table 7
Three Key Recommendations for Heart-Healthy Eating and Activity for Preschoolers

Recommendation	*Theme*	*Rationale*
Drink low-fat milk.	"If your age is over 2, Low-Fat milk's the one for you!"	As a single strategy for reducing dietary fat, use of non-fat milk alone may be sufficient to meet dietary fat and saturated fat goals for preschool children.
Eat vegetables, fruits, and grains every day.	"Vegetables, fruits and grains are best. (Can you pass the fiber test?)"[a]	Achieving "five-a-day" and fiber goals for children will help them replace excess fat and sugar calories with more nutritious foods achieve RDAs for more nutrients, and help them meet dietary fat goals as well.
Play hard	"Play hard to keep your body strong. Run and jump and skip along."	Children >2 yr old need 30 min active play each day. As energy expenditure increases; helps prevent obesity.

[a]Age + 5 g/d dietary fiber for kids over 2 yr.

themes for preschool diet and physical activity form the basis for educational messages for parents and teachers of young children as well. (Table 7).

Parents can be helped to establish diet and physical activity patterns that become healthy habits in the home—habits that promote health rather than increase risk of future chronic diseases such as obesity, CVD, diabetes, and cancer.

5.5. Principles of Prevention

The programs and activities described in this chapter include potential strategies that could be incorporated into an effective childhood obesity prevention campaign. Such strategies should benefit the vast majority of children as a population approach to the problem, including both those at increased risk of obesity as well as those at low risk. Based on scientific and behavioral considerations, childhood obesity prevention initiatives should:

- Begin early (at least by age 2 yr, if not earlier).
- Have no deleterious effects.
- Be relatively inexpensive.
- Benefit all children, even those at low risk of obesity.
- Have a strong child health education component (behavioral orientation).

Table 8
10 Steps to a Child-Healthy Diet

1	Keep up caloric intake for normal growth.
2	Keep total fat to 30% or less of calories (20–30%); keep saturated fat below 10% of calories; keep cholesterol intake below 300 mg/d.
3	Eat at least five vegetables and fruits each day (three vegetables; two fruits).
4	Keep up adequate calcium intake for bone health.
5	Increase carbohydrates to 50 to 55% of calories; eat a mix of starches, high fiber carbohydrates, and whole grains.
6	Eat at least "Age + 5" g of dietary fiber each day.
7	Increase protein from vegetable sources (soy, grains, etc.); decrease protein from animal sources.
8	Eat fish once a week.
9	Limit added sugar and salt.
10	Serve appropriate portion sizes for age and activity of child: smaller portions for preschool children; larger portions for very active children and teens.

The Academy of Pediatrics has also summarized recommendations for obesity prevention activities as part of health supervision by pediatricians and other health care providers. Additionally, it emphasizes the importance of advocacy and funding for childhood obesity prevention initiatives at all levels of government *(153)*.

- Include a child-healthy (and heart-healthy) diet at home and at preschool (Table 8).
- Include adequate daily physical activity ("An hour a day of active play").
- Limit inactivity (2 h TV per day).
- Involve the whole family (healthy home environments).
- Educate and involve parents, teachers, and older children as role models.
- Involve preschool centers in heart-healthy nutrition and physical activity initiatives.

6. SUMMARY AND RECOMMENDATIONS

In summary, prevention of childhood obesity should begin as early in life as possible—at least by age 2 to 3 yr. Preschool programs that include health education, daily physical activity, and a child-healthy diet could be an effective public health intervention strategy. Recommendations for dietary intake in early childhood must account for the child's unique growth and developmental patterns, specific nutrient requirements, vulnerability of sentinel nutrients, factors important to the establishment of healthy food acceptability, preference and intake patterns, and nutritional risk factors for disease during early childhood as well as later in life. All of these considerations are crucial in translating scientific findings into public policy.

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15 Obesity and Chronic Disease
Impact of Weight Reduction

Henry I. Frier and Harry L. Greene

KEY POINTS

- Obesity continues to rise and is most acute, as a public health issue, in pediatric and adolescent populations.
- Life expectancy, employing years-of-life-lost estimates, is drastically decreased.
- Based on NHANES III, approx 1 million overweight/obese teenagers suffer from metabolic syndrome.
- Weight reduction coupled with increases in physical activity remains the safest and most effective means to reduce insulin resistance.
- Obesity continues to be a major public health issue that must be addressed.

1. INTRODUCTION

"Opinion is a flitting thing but truth outlasts the sun."

—Emily Dickinson

It is impossible to pick up a newspaper or current magazine without being bombarded with the latest legal or scientific findings linking diet to obesity and disease. Diet, poor eating habits, and a sedentary lifestyle play a significant role in the health of individuals. Improvements in public health and efficiencies in the processed food delivery complex have eliminated most nutrient deficiencies in the United States and replaced them with diseases involving excess calories. *The Surgeon General's Report on Nutrition and Health* (1988) ranked the leading causes of death in the Unites States *(1)*, with several strongly related to diet (i.e., coronary heart disease [CHD], cancer, stroke, diabetes mellitus, and atherosclerosis). The relatively meek report, *Surgeon General's Call to Action To Prevent Overweight and Obesity 2001 (2)*, addresses the social and community aspects of this epidemic and is countered by the more strongly worded World Health Organization Technical Report *(3)*, which identifies specific food components that are directly related to obesity and disease. Despite the recommendations of the *Surgeon General's Report (1)* to maintain a desirable weight and a

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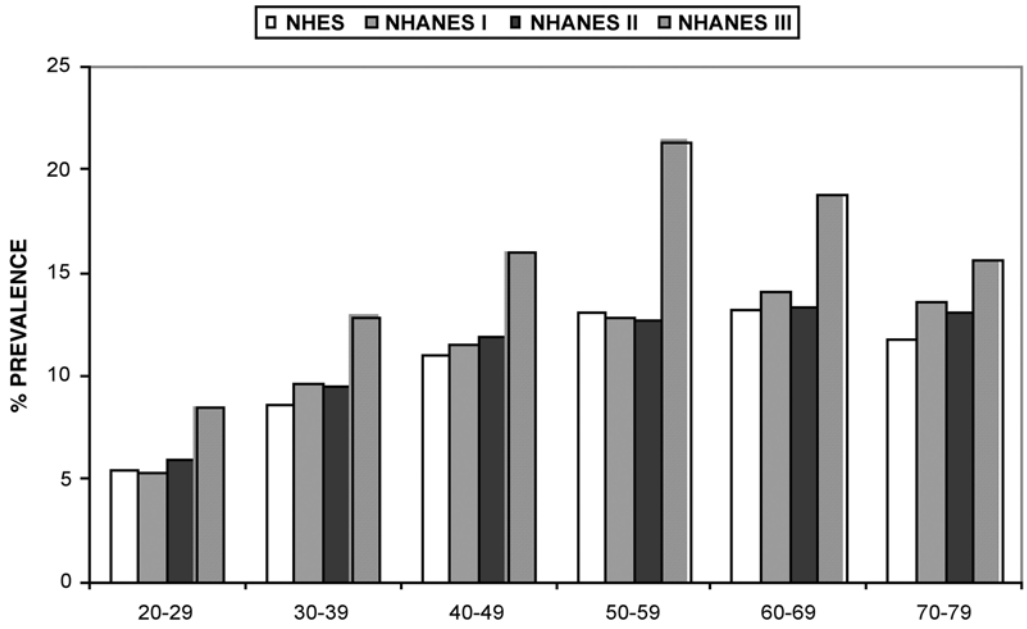

Fig. 1. Trends in class I obesity (BMI: 30–34.9 kg/m^2) at different age ranges (yr). NHES, National Health Examination Survey; NHANES, National Health and Nutrition Examination Survey. (Adapted from ref. *5*.)

caloric intake keeping with energy expenditure, Americans continue to experience increases in body weight- and obesity-related morbidity and mortality. The prevalence of weight problems and obesity continues to climb and is currently estimated to include 65% of the adult population *(4)*. The greatest increase was seen in those classified as obese, represented by the heaviest individuals (now estimated at 23%) with a body mass index (BMI) of greater than 30 (Fig. 1) *(5)*. The recently reported age-adjusted prevalence of 30.5% from the National Health and Nutrition Examination Survey (NHANES) of 1999–2000 demonstrates that this trend is continuing *(4)*. Of greatest concern is the increased incidence of obesity in children and adolescents. Evidence suggests that they will be the next generation of obese adults and will experience the accompanying disease risks. In a 1995 report, the incidence was estimated at 14%, using the 95th percentile of weight (Fig. 2) *(6)*. Newer estimates, based on examination data from the 1999–2000 NHANES estimated the prevalence at 15.5% for children ages 12 through 19 yr, 15.3% for children ages 6 through 11 yr, and 10.4% for children ages 2 through 5 yr *(7)*.

In 1995, the estimated annual direct cost of obesity and its related comorbidities was US$52 billion *(8)*. This accounted for 5.7% of the US national health expenditure. The authors again echoed the recommendations of the *Surgeon General's Report* to improve the diet and avoid weight problems and obesity as the most important step in the containment of rising health care costs.

The purpose of the earlier chapter, which appeared in the second edition, was to review the association between excess body weight and its major comorbid conditions, as well as to review the impact of weight reduction on improvement in obesity-related diseases. Five major comorbidities were discussed: type 2 diabetes mellitus (DM),

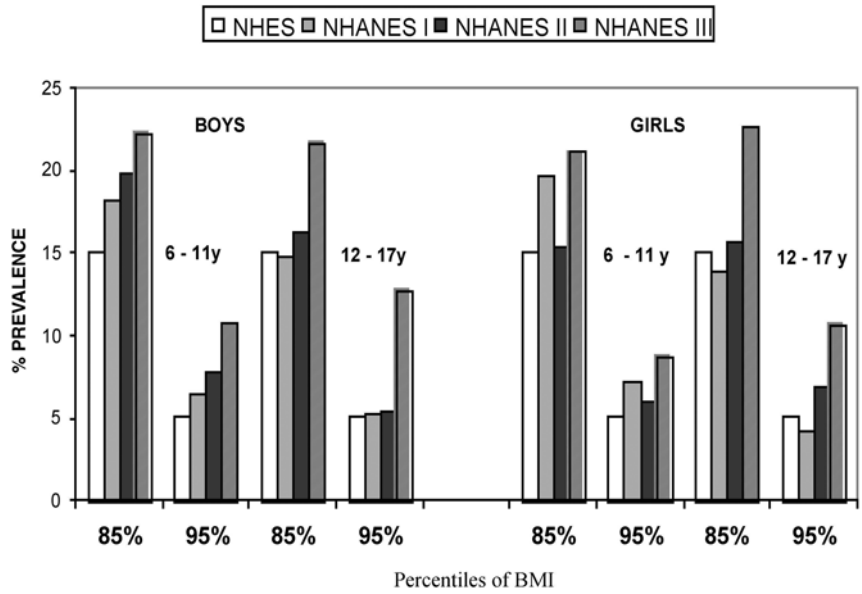

Fig. 2. Trends in obesity in boys and girls ages 6–11 and 12–17 yr. (Adapted from ref. *6*.)

hypertension (HT), cardiovascular disease (CVD), dyslipidemia, and certain types of cancer. This chapter is updated with the latest findings in specific areas and reviews the latest developments regarding metabolic syndrome and its implications for disease.

Obesity results from an imbalance in energy consumed and energy expended. It is believed that there is a permissive component that is genetic but is promoted by the societal and environmental changes occurring in contemporary life (i.e., dietary patterns and decreased physical activity). There is little doubt that a 5 to 10% decrease in body weight can significantly improve several risk factors associated with chronic disease *(9)*. However, is there an improvement in mortality associated with the reduction in risk factors? Intuitively, one would expect that reductions in the risk factors (i.e., blood lipids, blood pressure, and glycemic control) would improve the health of the population, thus improving mortality rates. Additionally, because the positive relationship of weight gain and mortality has been established *(10)*, logic would suggest that reversing this gain would decrease mortality.

Several recent reviews critically evaluated the epidemiological studies on the association of weight loss and increased longevity *(11–13)*. Williamson and Pamuk *(11)* concluded that the prospective studies reporting a positive benefit of weight loss on mortality were not justified by the data, whereas Andres et al. *(12)* concluded that moderate weight gain was associated with the lowest rates of all-cause mortality after adjusting for weight volition and smoking.

The majority of the studies evaluated by Williamson and Pamuk *(11)* may have been misleading because two key confounders (unintentional weight loss caused by an underlying illness and habitual smoking) were not considered *(11,13)*. Analyzing data as a percent of weight lost in a cohort from NHANES I, Williamson (while correcting for smoking and premature death *[13]*) found that a greater relative risk (RR) of death was present for both genders at each BMI category between 26 and 29 kg/m^2 but decreased for those individuals with a BMI less than 29 kg/m^2.

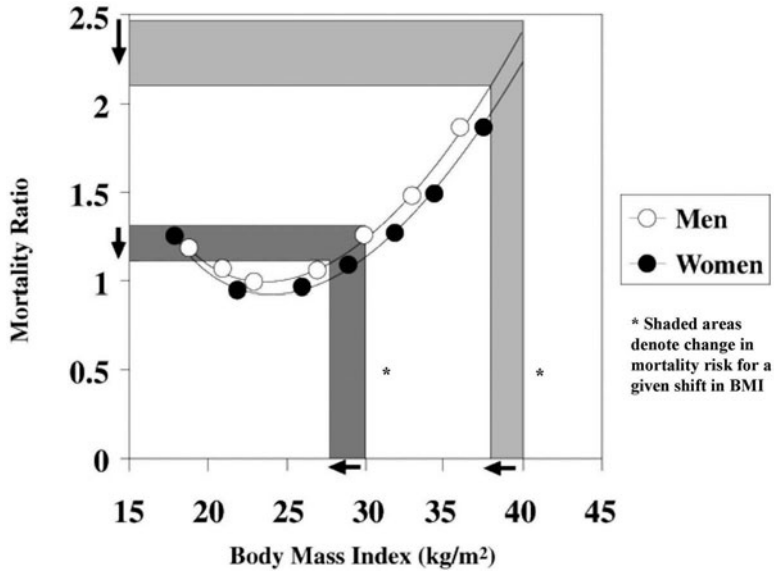

Fig. 3. Relationship between all-cause mortality and BMI. Theoretical changes in mortality risk with decreases in BMI are denoted by arrows. (Reproduced from ref. *15*.)

In contrast to the reports mentioned earlier on mortality are several studies supporting the role of weight loss in improving mortality risk. Lean et al. *(14)* retrospectively analyzed mortality data in diabetics and found that weight loss *per se* and its relationship with initial BMI actually improved survival. They estimated a 3- to 4-mo increased survival with each 1 kg of body weight lost. Goldstein *(15)* reviewed the available clinical studies reporting weight losses of 10% or less in obese individuals with either type 2 DM, HT, or hyperlipidemia and also concluded that modest weight loss decreased risk factors and, therefore, should increase longevity. Goldstein theorized that a decrease in BMI from 40 to 38 kg/m^2 would improve mortality risk by 12% and that decreasing the BMI from 30 to 28.5 kg/m^2 would improve mortality by 7.5% (Fig. 3, shaded areas; ref. *15*). More recently, Fontaine et al. *(16)*, employing years-of-life-lost estimates concluded that obesity, especially in younger age brackets, markedly lessens life expectancy. These data suggest that even the morbidly obese can reduce mortality risk with as little as a 5% weight reduction.

To understand the association between intentional weight loss and longevity, Williamson et al. *(17)* analyzed prospective data from overweight and obese women in the Cancer Prevention Study I. Exclusions were a BMI of 27 or less, smoking, and age older than 64 and younger than 40 yr. Women who had an obesity-related health condition and intentionally lost weight had a 20% lower all-cause mortality. Diabetes-associated mortality was also reduced by 44%, and CVD mortality was reduced by 9%. Weight loss of more than 9 kg in women without a pre-existing disease was associated with a 25% decrease in cancer and CVD mortality *(17)*.

The benefit of weight loss on mortality remains controversial. Several critical evaluations of the published observational data challenge the conventional wisdom that weight loss improves mortality. On the other hand, the weight of clinical trials (e.g., cholesterol reduction; *see* Subheadings 2.3. and 2.4.) and more recent observational studies *(16,17)* suggest otherwise.

2. OBESITY-RELATED HEALTH RISKS AND THE BENEFITS OF WEIGHT LOSS

2.1. Impaired Glucose Tolerance and Type 2 DM

The medical consequences of obesity can be classified as metabolic (e.g., DM and dyslipidemia) or mechanical (e.g., joint pain and osteoarthritis). The role of obesity in the development of the diabetic state is well-established. Type 2 diabetes, via impaired glucose tolerance (IGT) and insulin resistance (IR), is also related to the development of several additional risk factors (e.g., HT and dyslipidemia). This relationship plays a key role in the progression to increased risk of macro- and microvascular diseases such as kidney failure and blindness *(18)*. For example, Ohlson et al. *(19)* observed that obese middle-aged Swedish men with IGT were more likely to have both an increase in systolic blood pressure and an elevation in plasma triglycerides.

The prediabetic state is marked by an increased level of fasting-circulating insulin and a diminished response of peripheral insulin-sensitive tissues to oxidize and store glucose (IR) *(20)*. Increased circulating insulin in the obese individual is insufficient to maintain normal blood glucose because of increased hepatic glucose production coupled with a reduced glucose uptake by muscle and adipose tissue. The relation of adiposity to this pathological state is speculative. One early theory *(21)*, "the glucose fatty acid cycle," suggests that in obesity, an excess of circulating free fatty acids inhibit glucose uptake by muscle, inducing glucose intolerance. It is believed that an imbalance exists between rates of fatty acid esterification in adipose tissue and an accelerated glyceride breakdown in muscle caused by the insulin insensitivity of this tissue.

2.1.1. BMI and Diabetes Risk

Campbell and Gerich *(22)* measured both insulin sensitivity, by half-maximal glucose disposal rates (EC_{50}), and insulin responsiveness in subjects with varying BMI and normal glucose tolerance. They found that BMI must exceed a critical threshold before insulin action is impaired. Using a broken-line regression model, they estimated a breakpoint in BMI of 26.8 kg/m^2, which corresponds to 120% of ideal body weight. In a follow-up study, Campbell and Carlson *(23)* used similar techniques to compare normal lean subjects to obese type 2 diabetics (52 ± 2 yr; BMI of 28.1 ± 0.8 kg/m^2). The results strengthen the earlier findings of a relationship between increased BMI and decreased insulin sensitivities in type 2 diabetics. Additionally, hepatic glucose production was correlated with BMI only in the type 2 diabetics. These findings are consistent with the epidemiological data and the longheld clinical association between obesity and type 2 diabetes.

2.1.2. Visceral Adiposity and Diabetes Risk

Several lines of evidence suggest that increased visceral adiposity is more likely to be associated with abnormal glycemic control and subsequent development of diabetes than simply an increased body weight. Several prospective studies showed an increased relative risk of diabetes in both men and women with increased central (visceral) obesity. The Gothenburg studies *(24,25)* focused on the importance of central adiposity and the increased incidence of diabetes; both men and women had significant correlations with waist-to-hip ratio (WHR) and total body fat. Using a cohort from the Normative Aging Study, Cassano et al. *(26)* found a 2.4-fold increase in diabetic risk with increasing WHR when adjustments were made for age, BMI, and smoking. These authors

also found a positive association between blood glucose and abdominal fat that was independent of total body fat.

2.1.3. Metabolic Alterations and Insulin Resistance

Other metabolic alterations are commonly associated with obesity and IR. Dyslipidemia manifested as hypertriglyceridemia, decreased high-density lipoprotein cholesterol (HDL-C), increased very low-density lipoprotein (VLDL) triglyceride, and reduction in low-density lipoprotein (LDL) particle size (increased atherogenic potential) increases the risk for age-related CHD *(27)*. Although those with type 2 diabetes and good glycemic control have normal concentrations of LDL-C, the concentrations of fasting and postprandial triglyceride-rich particles (VLDL) are often elevated, and HDL-C levels are decreased *(28)*.

2.1.4. Weight Loss and Glycemic Control

Weight loss has been shown repeatedly to improve glycemic control in both obese diabetic and nondiabetic patients *(9,20)*. These improvements have been observed early during therapy, suggesting that the caloric reduction is the primary promoter for the observed changes. Wing et al. *(29)* attempted to separate the effects of caloric restriction and weight loss in obese patients with type 2 diabetes. These investigators used two levels of caloric restriction (400 vs 1000 kcal/d). At the point of an 11% weight loss, assessment of subjects showed that individuals consuming fewer calories per day had greater reductions in fasting blood glucose and greater insulin sensitivities. Therefore, the degree of calorie restriction, independent of the magnitude of weight loss, appeared to play a dominant role in improvements in glycemic control. Additional support for the primary role of calorie reduction is provided by a report of patients with type 2 diabetes who are undergoing gastric bypass surgery for weight loss *(30)*. These patients showed an early correction of hyperglycemia before the appearance of weight loss. The authors relate these changes to the immediate reduction of food intake following surgery. They were also able to rule out the interaction of more traditional treatment approaches such as exercise, sulfonylureas, and insulin, which were not part of the surgical recovery regimen.

2.1.5. Diabetes and CHD

Controversy exists regarding the association between CHD mortality and type 2 diabetes. Observational and prospective studies have not demonstrated a clear relationship between the hyperglycemia in type 2 diabetics and CHD *(28)*. However, several studies showed an association between glucose-intolerant populations and increased risk of CHD. Singer et al. *(31)* also found a significant relationship between hemoglobin A_1C (HbA_{1c}) and CVD in female survivors of the original cohort of the Framingham Heart Study. In addition, HbA_{1c} was strongly related to HT and the ratio of LDL-C to HDL-C. An analysis of the Framingham Offspring Study *(32)* found several associated metabolic abnormalities. For example, obesity was associated with HT and an elevation in fasting insulin, low HDL-C, elevated triglyceride concentrations, and a wide range of IGTs.

2.1.6. Weight Loss and Improvement in Risk Factors

Clinically, improvements in glycemic control accompanying body weight loss are also mirrored by improvements in plasma insulin concentrations and lipid profiles *(33)*. In type 2 diabetics, long-term weight loss ranging from 2.3 to 13.6 kg was associated with significant graded improvements in fasting blood glucose, HbA_{1c}, insulin, triglycerides, and HDL-C (Table 1). Weinsier et al. *(34)* found that 10 d of energy restriction (800 kcal/d) produced significant decreases in plasma total cholesterol, triglycerides,

Table 1
Metabolic Improvements in Patients With Type II Diabetes Following Weight Loss Over 1 Yr

	Before	*13.6 kg*	*6.9–13.6 kg*	*2.4–6.8 kg*	*0–2.3 kg*	*Weight gain*
RBW%	159	121[a]	143[a]	152[a]	157[b]	164[a]
HbA_1 (%)	9.7	7.1[b]	8.7[a]	9.8	10.4	10.6[c]
Glucose (mg/dL)	190	109[a]	162[b]	185	197	217[c]
Insulin (mU/dL)	20	2.9[a]	10.5[c]	11.8[c]	14.6	14.9
Triglyceride (mg/dL)	195	87[a]	155[b]	165[c]	175	204
HDL-C (mg/dL)	28.2	48.8[a]	41.3	40.3	38.4	39.7

p-values, [a]0.001, [b]0.01, [c]0.05. RBW%, relative body weight; HbA, hemoglobin A_{1C}; HDL-C, high-density lipoprotein-cholesterol

(Reproduced with permission from ref. *33*.)

LDL-C, and insulin (all at $p < 0.05$). Strong correlations existed between insulin concentrations and triglycerides. Several reports evaluated the efficacy of very low-calorie diets in patients with type 2 diabetes (40% or more above their ideal weight) for periods of 4–20 wk *(35–37)*. Serum cholesterol and triglycerides were significantly reduced and were associated with improvements in the fasting blood glucose and glycosylated hemoglobin. In one of these studies *(36)*, the improvements in the lipid profiles during active treatment were negated because of a partial weight regain. However, the regain in weight was not of sufficient magnitude to dampen the improvements observed in glycemic control and HDL-C.

2.2. Hypertension

Since the second edition of *Preventive Nutrition: The Comprehensive Guide for Health Professionals*, new guidelines have been issued from the National Heart, Lung, and Blood Institute *(38)* based on several large-scale clinical trials. The new classification of blood pressure reduces normal blood pressure to less than 120 mmHg systolic and less than 80 mmHg diastolic and provides for a new classification for pre-HT (systolic/diastolic: 120–139/80–89 mmHg). Stage 1 HT (140–159/90–99 mmHg) *(39)* afflicts more than 60 million Americans and, therefore, is the most common CVD in the United States. The regulation of blood pressure is complex and involves the interaction of a number of mechanical and hormonal control mechanisms *(40)*. HT from obesity is associated with both increased cardiac output and peripheral arteriolar resistance. It also is strongly associated with the male obesity pattern of upper body adiposity *(41)*.

2.2.1. Increased Risk of Hypertension From Obesity

Population comparisons indicate that HT is strongly associated with societies that also exhibit excess body weight. The Hypertension Detection and Follow-Up Study *(42)* found that 60% of HT participants were at least 120% of normal body weight, with BMIs of approx 27 kg/m^2. On further analysis, this study *(43)* found that in the 30- to 39-yr age group, a threefold greater incidence of HT occurred at body weights greater than or equal to 20% of normal. A 6-yr follow-up analysis of The Multiple Risk Factor Intervention Trial *(44)* reported a strong independent relation between increasing BMI and systolic and diastolic blood pressure. The most recent NHANES estimated that age-adjusted blood pressure increases progressively with increasing BMI in both genders (ref. *9* and Fig. 4).

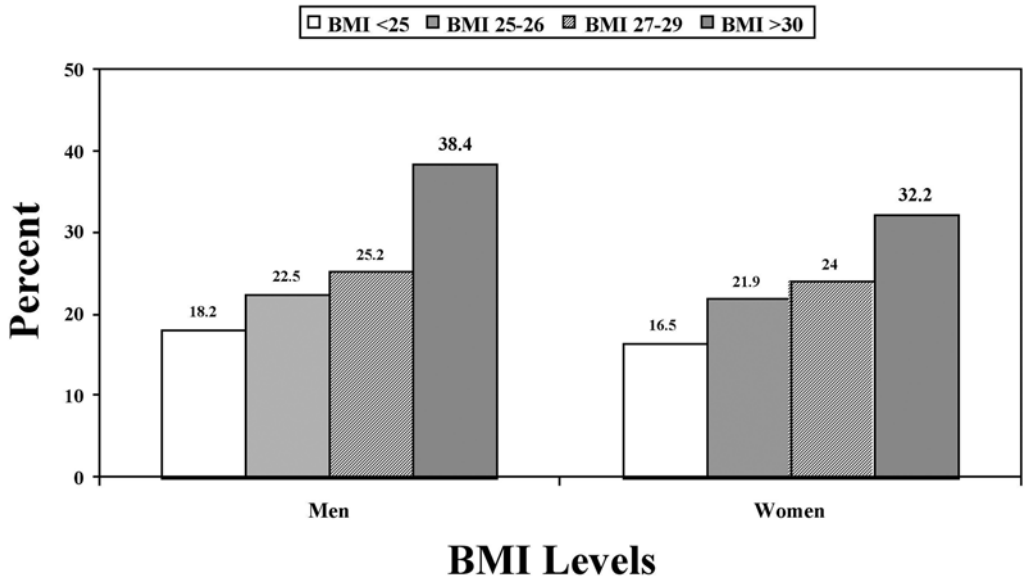

Fig. 4. Age-adjusted prevalence of HT (mean systolic blood pressure ≥140 mmHg and mean diastolic ≥90 mmHg) with increasing BMI in men and women. (Reproduced from ref. *9.*)

2.2.2. Effects of Weight Loss on Blood Pressure

The benefit of weight loss on blood pressure in obese patients with and without HT has been well-established *(9,20,45)*. A 10-kg body weight loss in obese subjects with HT was accompanied by a significant fall in both systolic and diastolic pressures and was associated with decreases in several cardiac parameters (e.g. heart rate, cardiac output, cardiopulmonary volume) *(46)*. A meta-analysis *(47)* found an average fall of 6 and 3 mmHg in systolic and diastolic blood pressures, respectively, with a 9-kg weight loss. Large-patient multicenter trials, the Trial of Nonpharmacologic Intervention in the Elderly (TONE) *(48)*, and the Trials of Hypertension Prevention (TOPH) *(49)*, demonstrated significant and independent effects of weight loss on blood pressure. Elderly individuals maintaining a weight loss of approx 4 kg over 30 mo had significantly lower clinical endpoints (HT, resumption of medication) than controls *(48)*. Subjects in TOPH who lost weight exhibited half the incidence of HT of the control group *(49)*.

The public health impact of a small downward shift in the population distribution of blood pressure was shown to reduce the community burden-related CVD risk *(50)*. In those ages 35–64 yr, a decrease of 2 mmHg in diastolic blood pressure was estimated to decrease the prevalence of hypertension by 17% and decrease the annual incidence of clinically relevant coronary artery disease and stroke by 6 and 14%, respectively *(50)*.

2.3. Cardiovascular Disease

2.3.1. Dyslipidemia

Analysis of the NHANES found that serum total cholesterol levels declined in the adult US population over the last 30 yr *(51)*. This finding suggests that the public health

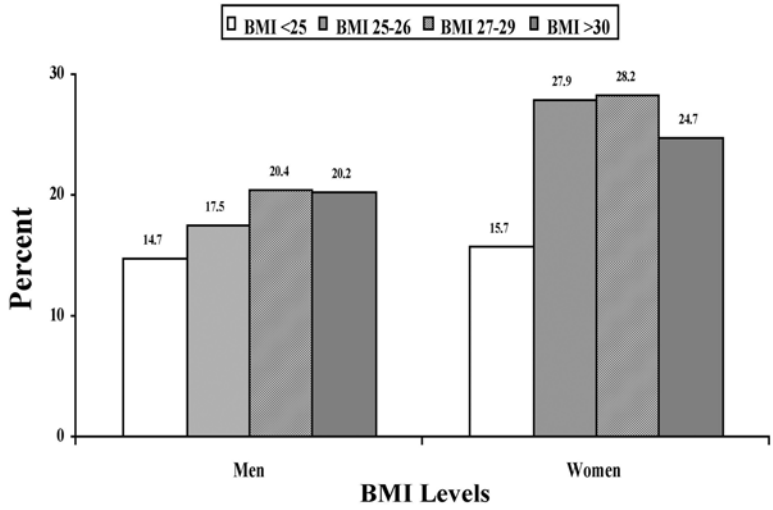

Fig. 5. Age-adjusted prevalence of high serum cholesterol (≥240 mg/dL) with increasing BMI in men and women. (Reproduced from ref. *9*.)

programs designed to lower serum cholesterol have been effective *(52)*. However, there is some debate regarding the relationship of serum total cholesterol and all-cause and cardiovascular mortality in women *(53,54)*.

2.3.1.1. Obesity and Cardiovascular Risk. The relationship between obesity, CVD risk factors, and increased risk of CHD and stroke has been demonstrated in several observational and prospective studies in both men and women *(55–61)*. Analysis of NHANES III data demonstrated the increasing prevalence of high serum total cholesterol (Fig. 5) and low HDL-C (Fig. 6) with increasing BMI *(9)*. Using data from NHANES II, Denke et al. *(58,59)* found that excess body weight resulted in deleterious changes in the lipoprotein profile of both men and women. A linear trend analysis showed that increased BMI was significantly related to elevations in serum triglycerides, total cholesterol, and LDL concentrations in both genders.

Several recent prospective analyses of the Nurses' Health Study *(55–57,60)* estimated the risk of CHD and stroke with increased body weight in more than 121,000 women. Similar relative risks of nonfatal myocardial infarction (MI) and fatal CHD in 8-*(60)* and 14-yr *(57)* follow-ups were 3.3 and 3.6, respectively, for a BMI of 29 kg/m^2 or greater. Of greater interest were the changes in relative risk with body-weight gain. Women who gained 8 to 10.9 kg from age 18 yr doubled their risk of CHD and nonfatal MI when compared to stable weight controls *(57)*. Higher WHR and waist circumference were independently associated with greater CHD risk in a cohort of women ages 40 to 65 yr in the Nurses' Health Study *(55)*.

2.3.1.2. Obesity and the Risk of Stroke. Examination of the risk of stroke with increasing BMI and weight change *(56)* in a cohort of registered nurses found that women with a BMI of at least 27 kg/m^2 had a significantly increased risk of ischemic stroke. Weight gain from age 18 yr was positively associated with ischemic stroke and was 1.7 times greater in women who gained 11 to 19 kg compared to stable weight controls.

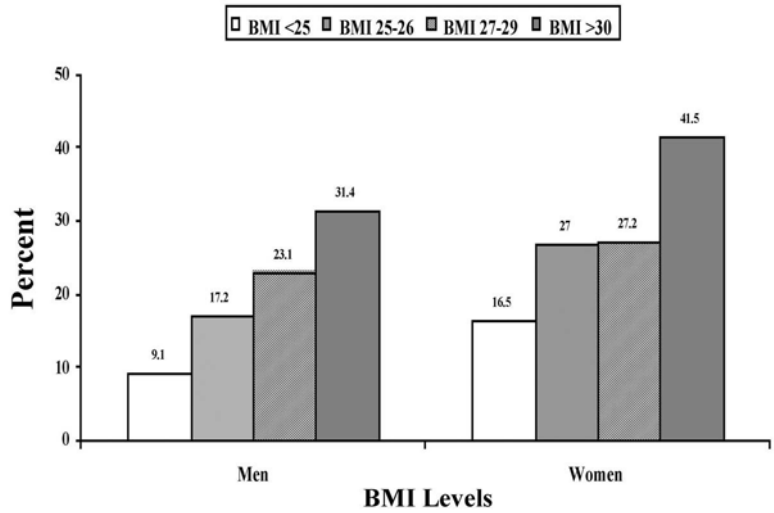

Fig. 6. Age-adjusted prevalence of low HDL-C (<35 mg/dL) with increasing BMI in men and women. (Reproduced from ref. *9*.)

Additional evidence for association of increases in body weight, blood lipids, blood pressure, and mortality was provided in the Framingham Heart Study *(61,62)*. Elevation in serum cholesterol, systolic and diastolic blood pressure, and blood glucose was positively associated with increasing weight gain in both men and women. Although total mortality did not increase until the highest quintile of BMI, CHD mortality increased linearly with increasing BMI. Two cross-sectional studies in Swedish women and men *(63,64)* provided additional support for this association. In women *(63)*, WHR was significantly and positively associated with the 12-yr incidence of MI, stroke, and death. Similar associations were present for Swedish middle-aged men *(64)*. Central obesity as measured by subscapular skinfold thickness in the Honolulu Heart Program was linearly related to the 12-yr incidence of CHD *(65)*. A twofold increase in risk was estimated in those men with the highest skinfold thickness compared to the lowest. Other CVD risk factors also increased with increasing tertiles of subscapular skinfold thickness (Table 2).

Modest reductions in body weight are paralleled by significant improvements in plasma lipids. Several reviews of the literature *(9,15,20,66)* found consistent improvements in plasma triglycerides, total cholesterol, and LDL-C. Changes in HDL-C varied among studies. Goldstein *(15)* reviewed six studies using various methods of weight reduction and found that HDL-C improved in half of these studies. The changes in HDL-C seem to be dependent on the magnitude of weight loss and gender. A 5% body weight loss was insufficient to raise HDL-C unless coupled with an extensive exercise program *(66)*. In a carefully controlled study to evaluate lipid and insulin changes in postmenopausal women, Weinser et al. *(34)* found significant improvements in total cholesterol, LDL-C, and triglycerides. No improvement was observed in either HDL-C or the LDL-to-HDL ratio with weight loss of 17% of initial body weight.

2.3.2. Thrombogenesis

The Northwick Park Heart Study *(67)* established a separate role for several hemostatic factors in ischemic heart disease. Increasing levels of clotting factor VII is an independent risk factor for CVD and is associated with obesity and elevated plasma

Table 2
Associated Risk Factor and Definite CHD With Increasing Tertiles of Subscapular Skinfold Thickness

	Tertile of subscapular skinfold		
Covariate	*Lowest*	*Middle*	*Highest*
BMI (kg/m^2)	21.2 (2.2)	24.1 (2.1)	26.3 (2.6)
Total cholesterol (mg/dL)	210.1 (37.7)	220.4 (37.2)	223.6 (38.1)
Glucose (mg/dL)	151.7 (52.6)	159.4 (57.2)	169.3 (62.9)
Triglycerides (mg/dL)	185.1 (168.8)	250.6 (211.2)	278.8 (218.3)
Incidence of CHD (no. of events)	97	161	190

Abbreviations: CHD, coronary heart disease; BMI, body mass index.
(Adapted from ref. *65*.)

triglycerides *(66)*. Landin et al. *(68)* measured components of the fibrinolytic system involved in the pathogenesis of CHD and atherosclerosis in matched women with gluteo-femoral or abdominal obesity. They found increased fibrinogen and plasminogen activator inhibitor (PAI)-1 in those obese women with a WHR of 0.8 or greater. PAI-1 was strongly correlated with fasting glucose and insulin levels and negatively correlated with insulin sensitivity (euglycemic clamp). A reduction in PAI-1 was demonstrated with energy restriction and weight reduction in obese patients *(67)*.

2.4. Metabolic Syndrome

The metabolic syndrome is characterized by individuals having three or more of a cluster of several risk factors based on IR; these risk factors were HT, IGT, hyperinsulinemia, increased levels of triglycerides, a decreased level of HDL-C, and obesity (central adiposity). Metabolic syndrome warrants a separate section for discussion because of the prevalence of this condition in the US population and the recommended and proven treatments that are available. Based on analyses of the NHANES III database, Ford et al. *(69)* and Park et al. *(70)* concluded that approx 20% of adults in the US population have this clustering of risk factors. Additionally, a staggering prevalence (28.7%; ref. *71*) of overweight adolescents have the metabolic syndrome. These prevalence figures equate to approx 1 million teenagers and 47 million adults.

Significant attention has been drawn to this syndrome with the latest National Cholesterol Education Program (NCEP) Adult Treatment Panel III recommendations *(72)*. The NCEP therapy strategy for reducing CVD risk is to lower LDL cholesterol but suggests that the full benefit of risk reduction can not be visualized without modification of the other metabolic risk factors attendant with this syndrome. It was concluded that the target therapy should also address the underlying insulin-resistant state, which is interrelated to abdominal obesity, elevated triglycerides, lowered HDL-C, and HT.

Weight reduction and increased physical activity remains the safest and most effective means to reduce insulin resistance for the overweight and obese individual *(72)*. Ample evidence exists to support this type of intervention. The Da Qing study *(73)* found significant reductions in the proportional hazards ratio for the incidence in the development of diabetes ranging from 31% for diet to 42% for diet and exercise combined. Poppitt et al. *(74)* placed overweight subjects with metabolic syndrome on low-fat complex carbohydrate diets for a 6-mo period. Body weights, BMI, and waist

circumference were significantly reduced, whereas LDL-C and triglycerides improved in a nonsignificant manner. This diet did not prevent the further fall in HDL-C as is typically seen with high-carbohydrate diets. The cumulative incidence rate of diabetes in subjects randomized to a lifestyle intervention with IGT was significantly reduced (58%) *(75)* compared to a control group receiving standard care. The lifestyle intervention provided individual guidance on increasing physical activity; approaches to reducing saturated fat intake and increasing consumption of fruits, vegetables, and whole grains; and advice on achieving at least a 5% weight loss. The Diabetes Prevention Program *(76)* found significantly greater reductions in the incidence of diabetes with lifestyle changes when compared to metformin.

2.5. Cancer

The rate of breast cancer has been increasing steadily in the United States and is the second leading cause of cancer death in women *(77)*. There is substantial evidence linking breast cancer to obesity, the attendant hormonal changes, and the causes of obesity itself (i.e., excess dietary fat and caloric intakes and decreased physical activity). There is, however, considerable debate over cause and effect.

2.5.1. Obesity and the Risk of Breast Cancer

The available case–control studies and large prospective studies are at variance over the association of BMI and breast cancer *(78)*. Of 13 case–control studies, 12 found a relative risk greater than 1 in postmenopausal women when the highest BMI (>32 kg/m^2) was compared to the lowest BMI (≤19 kg/m^2). However, large prospective studies did not support this relationship. The authors *(78)* cited a large Norwegian study in which the observed relative risk of 1.2 for a 10 kg/m^2 increase in Quetelet's index was considered insignificant given the large BMI change.

Reviewing the associations between site-specific cancers and several anthropometry measures, Ballard-Barbash *(79)* concluded that the observed associations for weight gain after age 18 yr and central body fat exhibit stronger associations than weight or BMI. Because developed societies have a more sedentary lifestyle and a higher caloric intake, weight gain and increased fat mass, especially visceral, are better predictors of breast cancer. Most—but not all—studies have shown a doubling of breast cancer risk with expanding stores of visceral fat in postmenopausal, but not premenopausal, women *(79)*.

As stated earlier, obesity can be caused by diet (excess energy intake) and/or decreased physical activity. The evidence for the role of diet is beyond the scope of this chapter, but the controversy that exists is worth mentioning. Hunter and Willett *(78)* critically evaluated the strength of the studies implicating the amount of dietary fat as a major factor in breast cancer incidence. The strongest evidence was produced by studies based on national food disappearance estimates and those based on dietary recall. In contrast, several large prospective studies, which are not subject to recall bias, found no relation to breast cancer (RR ≤ 1) when the highest deciles of total fat intake were compared to the lowest. A recent analysis of the Nurses' Health Study *(80)* found no difference in relative risk for breast cancer in subjects consuming 20% dietary fat compared to those consuming 30 to 35%. relative risk did not differ for type of fat consumed.

A central debate also concerns the relative carcinogenic effect of individual fatty acids vs the energy content of fat intake. Excess fat calories could promote primary

breast cancer by expanding centrally located adipose tissue, which in turn could increase the levels of circulating estrogens (higher conversion of adrenal androgens to estrone in female adipose tissue). The increased levels of estrogens have been implicated in the etiology of some breast and reproductive cancers (i.e., endometrial cancer) *(81,82)*. Recent studies have also implicated insulin and insulin-like growth factors, which are elevated in obesity, as promoting tumor growth directly or indirectly by their actions on estrogen and estrogen receptors *(79)*.

The evidence for a relationship between specific fatty acids or classes of triglycerides and breast cancer is equivocal. In a pooled analysis of case–control studies, the overall association of breast cancer with total fat was related to saturated fat intake. However, a pooled analysis of several large prospective studies found no relation of an increased risk *(83)*. Case—control and prospective cohort studies failed to find a relationship with breast cancer *(83)* for specific fatty acids, that is, long-chain n-3 fatty acids and 18:2 n-6 (linoleic acid).

2.5.2. Breast Cancer Survival and Obesity

Once breast cancer is diagnosed, women with higher BMIs exhibited poorer survival rates and increased recurrence of disease. In large-population cohorts, obese women with stage I or II disease had an increased risk of dying from breast cancer (70 and 40%, respectively *[79]*). Obesity is also associated with more advanced cancers at the time of diagnosis. In Dutch women, a greater incidence of metastatic disease in auxillary nodes was associated with significantly heavier body weights in the 50- to 69-yr age group *(77)*.

The data suggest that adult weight gain, whatever the cause, is associated with greater risks of breast cancer and that the extent of the disease is more highly pronounced in patients with the highest quintile of BMI.

2.5.3. Weight Loss and Cancer

No prospective human trials have been conducted for to demonstrate a reduction in risk of cancer from weight loss maintenance. However, Calle et al. *(84)*, in a prospectively studied cohort, recently reported a significant association between weight problems and obesity and risk of death from all cancers. These authors estimated that with maintenance of a normal body weight, 90,000 deaths could be prevented in the United States each year.

3. INTERVENTION THERAPIES: MANAGEMENT OF OBESE PATIENTS

A review of clinical studies concerning diet and diet plus increased physical activity demonstrated successful 1- to 2-yr weight loss ranging from 5 to 13% *(9)*. In these studies, reductions in plasma lipids ranged from 0 to −18% for total cholesterol, −2 to −44% for triglycerides, −2 to −22% for LDL-C, and −7 to 27% for HDL-C.

Significant reductions in diameter stenosis of coronary arteries occurred in patients who made intensive lifestyle changes (diet, physical activity, stress management, and smoking cessation) when compared to controls who did not make lifestyle changes (−5.3 vs 2.7%) *(85)*. Associated risk factors, BMI, serum total cholesterol, and LDL-C were significantly reduced. A 5-yr follow-up of these patients *(86)* showed continued improvement in regression of stenosis in the intervention group and worsening of the stenosis in the control group (−7.9 vs 27.7%).

A 10-wk multicenter trial *(87)* compared a prepackaged meal program based on the National Academy of Science-National Research Council (NAS-NRC) dietary recommendations to a self-selection diet based on the American Heart Association (AHA) Step I/II diets in 560 patients suffering from dyslipidemia, HT, or diabetes. Weight loss was significant for both groups (−4.7 kg for the NAS-NRC diet and −3.2 kg for the AHA diet) and was accompanied by significant reductions in major CVD risk factors (blood pressure, total cholesterol, glycemic control, and blood lipids). Similar findings in key risk factors occurred in a group of patients identified with acute MI or unstable angina *(88)*. After 1 yr, those patients randomized to a low-fat high-fruit, grain, and vegetable diet lost 6.3 kg of body weight and had significantly decreased cardiac events and mortality when compared to those patients in a control group, who were simply advised to follow a low-fat diet (*see* Chapter 12).

Improvement in health risk associated with long-term weight loss was studied by Ditschuneit et al. *(89)* in a randomized, controlled trial. These authors reported on a 2-yr follow-up of 100 obese patients (average BMI: 33 kg/m^2). Average weight loss of patients randomized to a traditional low-calorie diet group was 1.3 kg, with no improvements in markers of disease risk over the first 3 mo. By contrast, the group receiving the same prescribed calorie intake but that had two meals replaced with a Slim·Fast® liquid meal replacement lost an average of 7.1 kg. These patients showed significant improvements in blood pressure, plasma insulin, glucose, and triglyceride concentrations. A 24-mo follow-up of patients replacing an average of one meal per day with a Slim·Fast meal replacement demonstrated continued improvement in these biomarkers, with associated weight losses of 5 to 11.5% of their initial body weight. The authors followed these patients for another 2 yr *(90)* and found sustained weight loss and maintenance of the improvements in biomarkers.

The use of gastric bypass surgery *(30)* as a primary method for weight loss (10 yr) in morbidly obese patients is also instructive. In addition to the improvements in glycemic control, the sustained weight loss also corrected or alleviated a number of other comorbid conditions associated with obesity (e.g., HT, sleep apnea, arthritis, cardiopulmonary failure, and infertility).

4. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

The short-term and intermediate benefits of weight loss in the obese have been well-established *(9)*. Improvements in glycemic control in patients with type 2 diabetes occur early in the weight-loss regimen and mostly result from a reduction in calories ingested *(29,30)*.

Weight losses of 5 to 15% of initial body weight are beneficial for the reduction of CVD risk factors *(66)*. Cardiac patients making major lifestyle changes showed significant regression in aortic stenosis and cardiac events *(85–88)*. A program of reduced dietary fat intake, smoking cessation, stress management, and increased physical activity were essential to the improvements observed. It is difficult to isolate the individual impact that each of these changes had; however, the decrease in BMI in these patients was consistent with improvements in several of the risk factors.

Although it is obvious that weight loss will reduce risk factors for disease, the relationship to mortality remains tentative. The published conclusions discussed earlier in this chapter are troublesome because they suggest that the greater majority of individuals who

attempt weight loss may be at an increased risk for all-cause mortality. Furthermore, they imply that the assumption that weight loss improves the risk of mortality may not be as clear-cut as a simple reduction of risk factors *(91)*.

However, insight can be gained from clinical trials demonstrating improvements in CHD mortality with reductions in blood lipids. In a review of 22 clinical trials on cholesterol reduction *(92)*, there was a 23% risk reduction in CHD events (nonfatal MI and cardiac death). A meta-analysis of six primary prevention trials found mortality from CHD tended to be lower in men receiving interventions to reduce cholesterol. When analyzed separately, a significant decrease in mortality was found in each study *(93)*. The Scandinavian Simvastin Survival Study *(94)* showed significant improvement in lipid profiles in patients taking the drug. During 6 yr of follow-up, there were significantly more coronary deaths in the placebo group compared to the Simvastin group (189 vs 111, RR of 0.58). Therefore, the reduction of blood lipids that occurs with weight loss should provide similar changes in CHD risk.

Williamson's *(17)* 12-yr prospective study on weight loss and mortality added significantly to the expectation that weight loss, via improvements in risk factors, will improve mortality. Women with obesity-related comorbidities showed significant reductions in all-cause and diabetes-related mortality with weight loss. Preliminary evidence from this study suggest the same results for men *(9)*. Although it is recognized that there is limited data supporting improved mortality rates with weight loss *(95)*, the positive changes in biomarkers for disease risk justifies the public health objective to reduce the weight of the nation.

Recently, the National Institutes of Health *(9)* created a panel to assess the scientific literature regarding the severity of the medical problem and the benefits to weight loss. Based on the evidence, the panel concluded that obesity is a major public health problem that can, and should be, addressed. The panel further established the evidence-based guidelines to help physicians and associated health professionals manage the overweight and obese patient *(9)*.

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V Bone Diseases

16 Osteoarthritis

Role of Nutrition and Dietary Supplement Interventions

Timothy E. McAlindon

KEY POINTS

- Although there are numerous mechanisms by which micronutrients can be expected to influence the development of osteoarthritis (OA), there has been insufficient research to draw definitive conclusions.
- The strongest evidence points to a possible protective effect of vitamin D in the development of hip OA and the progression of knee OA.
- One observational study suggested a protective effect of vitamin C for progression of knee OA. Because intake of vitamin E and β-carotene bore no relationship to OA incidence or progression in that study, the mechanism of benefit of vitamin C may be mediated through nonantioxidant properties.
- Clinical trials of vitamins E, C, and A and selenium have produced negative or inconsistent results.
- There have been numerous positive clinical trials of glucosamine and chondroitin products for OA. Issues of biological plausibility, publication bias, and methodological problems cloud interpretation of these trials.

1. INTRODUCTION: OSTEOARTHRITIS

Osteoarthritis (OA) is the most common form of joint disease and is a leading cause of disability in the elderly. The disease commonly affects the hands, spine, knees, and hips. Multiple joints may be affected in any one individual. OA is strongly associated with increasing age and has been estimated to affect between 2 and 10% of all adults *(1,2)*. It is responsible for some 68 million days of work loss per year and more than 5% of the annual retirement rate *(3)*. Furthermore, OA is the most frequent reason for joint replacement, at a cost to the community of billions of dollars per year *(4)*.

Focal cartilage damage is the central feature of OA *(5)*. This may range in severity from minor surface roughening to complete erosion of cartilage. The process is generally

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noninflammatory and is often described as being "degenerative" or caused by "wear-and-tear." The cartilage damage usually is accompanied by some form of "reaction" in the surrounding bone. Osteophytes are an early bony response to cartilage damage. These consist of outgrowths of bone from the peripheral margin of the joint. They may confer some protection to an OA joint by reducing instability *(6)*. The trabeculae in the bone adjacent to an area of OA cartilage become thickened and are susceptible to microscopic fracturing. This gives rise to the appearance of "subchondral sclerosis" on a radiograph. In severe disease, particularly where full thickness cartilage has occurred, circumscribed areas of bony necrosis may develop in the subchondral bone. These may be filled with marrow fat or with synovial fluid that has tracked from the joint space through the cartilage defect into the subchondral bone. They give rise to the radiographical appearance of cysts. Ultimately, the subchondral bone itself may be eroded or may collapse. In some cases, a low-level synovitis develops because of the presence of crystal or other cartilaginous "detritus," which may contribute to damage in the joint.

Biochemically, cartilage consists of a network of collagen fibrils (predominantly type II), which constrain an interlocking mesh of proteoglycans that resist compressive forces through their affinity for water. The tissue is relatively avascular and acellular. Turnover in healthy cartilage is slow and represents a balance between collagen and proteoglycan synthesis and degradation by enzymes such as metalloproteinases. In early OA, the chondrocytes (cartilage cells) proliferate and become metabolically active. These hypertrophic chondrocytes produce cytokines (e.g., interleukin [IL]-1, tumor necrosis factor [TNF]-α), degradative enzymes (e.g., metalloproteinases), and other growth factors. Proteoglycan production is increased in early osteoarthritis but falls sharply at a later stage when the chondrocyte "fails."

2. RISK FACTORS FOR OSTEOARTHRITIS

The striking relationship of OA with increasing age and heavy physical work has led to its characterization as a degenerative or wear-and-tear disorder *(1)*. In fact, this paradigm is inaccurate, because a variety of mechanical, metabolic, and genetic disorders may lead to OA *(7)*. Thus, there is increasing recognition that OA should be regarded as joint failure, analogous to any other organ failure *(8)*. Established risk factors for OA are listed in Table 1.

3. OSTEOARTHRITIS AND DIET

There is enormous public interest in the relationship between diet and arthritis. Questions regarding nutrition and supplements are among the most frequent posed to physicians by people with OA. Speculative lay publications on this subject abound, and health food stores are full of nutritional supplements touted for their putative ability to help those who suffer from arthritis *(9)*.

In contrast, there has been relatively little focus in traditional scientific studies on the relationship between nutritional factors (other than obesity) and OA. Furthermore, the traditional physician stance regarding this issue has been to assert the lack of evidence supporting any association between diet and OA. This gulf between the levels of interest among scientists and among the general public is surprising given the large numbers of studies regarding osteoporosis, another widespread age-related skeletal disorder, which have shown widely accepted associations with dietary factors. However,

Table 1
Factors Associated With Osteoarthritis

Constitutional factors
Increased age
Female gender
Obesity
Mechanical factors
Heavy/repetitive occupations
Heavy physical activity
Major joint injury
Endocrine factors
Hemachromatosis
Genetic factors
Mutations in the type II collagen gene

a more important reason to study the relationship between dietary factors and OA is that there are many mechanisms by which certain micronutrients can be hypothesized to influence OA processes. Furthermore, there have been several recent studies that have shown apparent effects of various micronutrients on the natural history of this disorder.

4. MECHANISMS THROUGH WHICH MICRONUTRIENTS CAN BE HYPOTHESIZED TO INFLUENCE OSTEOARTHRITIS PROCESSES

4.1. Antioxidant Effects

There is considerable evidence that continuous exposure to oxidants contributes to the development or exacerbation of many of the common human diseases associated with aging *(10)*. Such oxidative damage accumulates with age and has been implicated in the pathophysiology of cataracts *(11)*, coronary artery disease (CAD *[12]*), and certain forms of cancer *(13)*. As the prototypical age-related degenerative disease, OA may also be, in part, a product of oxidative damage to articular tissues.

Reactive oxygen species are chemicals with unpaired electrons. These are formed continuously in tissues by endogenous and some exogenous mechanisms *(10)*. For example, it has been estimated that 1–2% of all electrons that travel down the mitochondrial respiratory chain leak, forming a superoxide anion ($O_2^{\bullet -}$) *(14)*. Other endogenous sources include release by phagocytes during the oxidative burst, generation by mixed-function oxidase enzymes, and hypoxia-reperfusion events *(15)*. Reactive oxygen species are capable of causing damage to many macromolecules, including cell membranes, lipoproteins, proteins, and DNA *(16)*. Because these reactive oxygen species are identical to those generated by irradiation of H_2O, "living" has been likened to being continuously irradiated *(10)*.

Furthermore, there is evidence that cells within joints produce reactive oxygen species and that oxidative damage is physiologically important *(17)*. In laboratory studies, animal and human chondrocytes were found to be potent sources of reactive oxygen species *(17,18)*. Hydrogen peroxide production was demonstrated in aged human chondrocytes after exposure to the proinflammatory cytokines, IL-1, and TNF-α, and also was observed in live cartilage tissue *(19)*. Superoxide anions were shown to adversely affect collagen

structure and integrity in vitro and appear to be responsible in vivo for depolymerization of synovial fluid hyaluronate *(18–21)*. Furthermore, patients with cartilage and/or meniscal damage associated with OA had increased levels of reactive oxygen species and nitric oxide compared with controls *(22)*.

In fact, the human body has extensive and multilayered antioxidant defense systems *(10)*. Intracellular defense is primarily provided by antioxidant enzymes, including superoxide dismutase, catalase, and peroxidases. In addition to these enzymes, there are a number of small molecule antioxidants that play an important role, particularly in the extracellular space, where antioxidant enzymes are sparse *(23)*. These include the micronutrients α-tocopherol (vitamin E), β-carotene (a vitamin A precursor), other carotenoids, and ascorbate (vitamin C). The concentrations of these antioxidants in the blood primarily are determined by dietary intake. The concept that micronutrient antioxidants might provide further defense against tissue injury when intracellular enzymes are overwhelmed has led to the hypothesis that high dietary intake of these micronutrients might protect against age-related disorders. Because higher intake of dietary antioxidants appears to be beneficial regarding outcomes such as cataract extraction and CAD *(11–13,24)*, it is also plausible that they may confer similar benefits for OA.

4.2. Effects on Collagen Metabolism

In addition to being an antioxidant, vitamin C plays several functions in the biosynthesis of cartilage molecules. First, through the vitamin C-dependent enzyme lysylhydroxylase, vitamin C is required for the posttranslational hydroxylation of specific prolyl and lysyl residues in procollagen—a modification essential for stabilization of the mature collagen fibril *(11–13,24–26)*. Vitamin C also appears to stimulate collagen biosynthesis by pathways independent of hydroxylation, perhaps through lipid peroxidation *(27)*. In addition, by acting as a carrier of sulfate groups, vitamin C participates in glycosaminoglycan synthesis *(28)*. Therefore, relative deficiency of vitamin C may impair not only the production of cartilage but also its biomechanical quality.

The results of in vitro and in vivo studies are concordant with this possibility. Studies of adult bovine chondrocytes showed that addition of ascorbate to the tissue culture results in decreased levels of degradative enzymes and increased synthesis of type II collagen and proteoglycans *(28,29)*. Peterkovsky et al. *(30)* observed decreased synthesis of cartilage collagen and proteoglycan molecules in guinea pigs deprived of vitamin C. They also reported high levels of insulin-like growth factor (IGF)-1 binding proteins, which normally inhibit the anabolic effects of IGF-1, a potent growth factor. This suggests that vitamin C may also influence growth factors through pathways that remain to be elucidated.

4.3. Effects on Chondrocyte Metabolism

Vitamin D might have direct effects on chondrocytes in OA cartilage. During bone growth, vitamin D regulates the transition from growth plate cartilage to bone. Normally, chondrocytes in developing bone lose their vitamin D receptors with the attainment of skeletal maturity. However, it recently has become apparent that the hypertrophic chondrocytes in OA cartilage can redevelop vitamin D receptors *(31)*. These chondrocytes are metabolically active and appear to play an important role in the pathophysiology of OA *(32)*. Although this evidence is indirect, it raises the possibility that vitamin D may influence the pathological processes in OA through effects on these cells.

4.4. Effects on Bone

Reactive changes in the bone underlying and adjacent to damaged cartilage are an integral part of the OA process *(33–39)*. Sclerosis of the underlying bone, trabecular microfracturing, attrition, and cyst formation all are likely to accelerate the degenerative process as a result of adverse biomechanical changes *(40,41)*. Other phenomena, such as osteophytes (bony spurs), may be attempts to repair or stabilize the process *(6,42)*. It also has been suggested that bone mineral density may influence the skeletal expression of the disease, with a more erosive form occurring in individuals with "softer" bone *(43)*. Although some cross-sectional studies suggested a modest inverse relationship between OA and osteoporosis, recent prospective studies suggested that individuals with lower bone mineral density are at increased risk for OA incidence and progression *(44)*. The idea that the nature of bony response in OA may determine outcome was advanced further by the recent demonstration that patients with bone scan abnormalities adjacent to an OA knee had a higher rate of progression than those without such changes *(45)*.

Normal bone metabolism is contingent on the presence of vitamin D, a compound that is derived largely from the diet or from cutaneous exposure to ultraviolet light. Suboptimal vitamin D levels may have adverse effects on calcium metabolism, osteoblast activity, matrix ossification, and bone density *(46,47)*. Therefore, low tissue levels of vitamin D may impair the ability of bone to respond optimally to pathophysiological processes in OA and predispose to disease progression.

4.5. Anti-Inflammatory Properties

Vitamin E has diverse influences on the metabolism of arachadonic acid, a proinflammatory fatty acid found in all cell membranes. Vitamin E blocks formation of arachidonic acid from phospholipids and inhibits lipoxygenase activity, although it has little effect on cyclooxygenase *(48)*. Therefore, it is possible that vitamin E reduces the modest synovial inflammation that may accompany OA.

5. STUDIES OF THE EFFECTS OF NUTRIENTS IN OSTEOARTHRITIS

5.1. Obesity and Osteoarthritis

People who are overweight are at considerably increased risk for the development of OA in their knees and may also be more susceptible to both hip and hand joint involvement *(49)*. Because overweight individuals do not necessarily have increased load across their hand joints, investigators have wondered whether systemic factors, such as dietary factors or other metabolic consequences of obesity, may mediate some of this association. Indeed, early laboratory studies using strains of mice and rats appeared to suggest an interaction between body weight and genetic factors and diet, although attempts to demonstrate a direct effect of dietary fat intake proved inconclusive *(50,51)*. The fact that adipocytes share a common stem cell precursor with connective tissue cells such as osteoblasts and chondrocytes prompted investigation into the possibility that the metabolic milieu might influence their phenotypic differentiation *(52)*. Indeed, fat and fatty acids have been associated with OA changes in joints and can influence prostaglandin and collagen synthesis in vitro *(52,53)*. Preliminary evidence also suggests that leptin, an adipose tissue-derived hormone, may have anabolic effects in OA cartilage *(54)*.

Table 2
Epidemiological Studies of Nutritional Factors and Osteoarthritis (OA)

Author	*Nutritional factors studied*	*Outcome variables*	*Summary*
McAlindon *(57)*	Dietary antioxidant intake	Knee OA incidence and progression	Vitamin C appeared protective for OA *progression* but not incidence.
McAlindon *(71)*	Vitamin D: dietary and serum	Knee OA incidence and progression	Vitamin D appeared protective for *progression* but *not incidence*.
Baker *(61)*	Vitamin C	Knee OA pain	Particiants with low vitamin C intake (especially smokers) had more knee pain.
McLauglin *(73)*	Vitamin D and parathyroid hormone	Knee OA and progression	No effect observed.
Lane *(72)*	Vitamin D: serum	Hip OA incidence and progression	Vitamin D appeared protective for hip OA incidence and progression.

5.2. The Case for Vitamin C and Other Antioxidant Micronutrients

5.2.1. Animal Studies

OA can be induced in animals by various surgical procedures. Schwartz and Leveille *(55)* treated guinea pigs prior to such surgery with either a high (150 mg/d) or low (2.4 mg/d) dose of vitamin C. Guinea pigs treated with the higher dose of vitamin C (which would correspond to at least 500 mg/d of vitamin C in humans) showed "consistently less severe joint damage than animals on the low level of the vitamin." Features of OA were significantly less frequent in the animals treated with the high dose of vitamin C. Similar findings were reported by Meacock et al. *(56)* in another surgically induced guinea pig model of OA. In this study, after the surgical procedure, they supplemented the feeds of half of the animals with vitamin C. They reported, "Extra ascorbic acid appeared to have some protective effect ($p = 0.008$) on the development of spontaneous [osteoarthritis] lesions..."

5.2.2. Epidemiological Studies (Table 2)

We investigated the association of self-reported dietary intake of antioxidant micronutrients among participants followed longitudinally in the Framingham Knee OA Cohort Study *(57)*, a population-based group derived from the Framingham Heart Study Cohort (Table 2). Participants had knee X-rays taken at a baseline examination performed during1983–1985 and had follow-up X-rays approx 8 yr later, during 1992–1993. Knee OA was classified using the Kellgren and Lawrence grading system *(58)*. Knees

without OA at baseline (Kellgren and Lawrence grade of 1 or less) were classified as incident OA if they changed to grade 2 or greater by follow-up. Knees with OA at baseline were classified as progressive OA if their grade increased by 1 or more.

Nutrient intake, including supplement use, was calculated from dietary habits reported at the midpoint of the study using a food-frequency questionnaire. In our analyses, we ranked micronutrient intake into gender-specific tertiles and specifically looked to determine whether higher intakes of vitamin C, vitamin E, and β-carotene were associated with reduced incidence and reduced progression of knee OA, compared with a panel of nonantioxidant control micronutrients. The lowest tertile for each dietary exposure was used as the reference category. Odds ratios (ORs) were adjusted for age, gender, body mass index (BMI), weight change, knee injury, physical activity, energy intake, and health status.

Six-hundred forty participants (mean age: 70.3 yr) had complete assessments. Incident and progressive knee OA occurred in 81 and 68 knees, respectively. We found no significant association of incident radiographic knee OA with any micronutrient (e.g., adjusted OR for highest vs lowest tertile of vitamin C intake =1.1, 95% confidence interval [CI] 0.6–2.2). However, *for* progression of radiographic knee OA, we found a threefold reduction in risk for those in the middle and highest tertiles of vitamin C intake (adjusted OR for highest vs lowest tertile = 0.3, 95% CI 0.1–0.6). It is notable that those in the highest tertile for vitamin C intake also had reduced risk of developing knee pain during the course of the study (OR= 0.3, 95% CI 0.1–0.8). Reduction in risk of progression was also seen for β-carotene (OR= 0.4, 95% CI 0.2–0.9) and vitamin E but was less consistent because the β-carotene association diminished substantially after adjustment for vitamin C, and the vitamin E effect was seen only in men (OR = 0.07, 95% CI 0.01–0.6). No significant associations were observed for any of the micronutrients among the nonantioxidant panel.

Therefore, this study did not support the hypothesis that diets high in antioxidant micronutrients reduce the risk of incident knee OA. However, the data suggest that some of these micronutrients, particularly vitamin C, may reduce the risk of OA progression among those who already have some radiographical changes.

If antioxidants are indeed protective for individuals with OA, then why does the effect appear to be confined to those with existing radiographical changes? One possible explanation relates to differences in the intra-articular environment between healthy and OA knees. For example, several pathological mechanisms—including raised intra-articular pressure *(16)*, low-grade inflammation *(59)*, and increased metabolic activity *(15)*—increase the opportunity for oxidative damage in an a knee with OA. Therefore, antioxidants could have a greater role in preventing progression rather than incidence, which can result from a variety of nonmetabolic insults, such as knee injury *(60)*.

Another important observation in this study was that the effect of vitamin C was stronger and more consistent than for those of β-carotene and vitamin E. Vitamin C is a water-soluble compound with a broad spectrum of antioxidant activity resulting from its ability to react with numerous aqueous free radicals and reactive oxygen species *(10)*. The extracellular nature of reactive oxygen species-mediated damage in joints and the aqueous intra-articular environment may favor a role for a water-soluble agent such as vitamin C rather than for fat-soluble molecules like β-carotene or vitamin E. In addition, it has been suggested that vitamin C may regenerate vitamin E at the water–lipid interface by reducing α-tocopherol radical back to α-tocopherol. However, the issue of whether this occurs in vivo appears controversial. An alternative explanation is that the protective

effects of vitamin C relate to its biochemical participation in the biosynthesis of cartilage collagen fibrils and proteoglycan molecules, rather than its antioxidant properties.

Recently, Baker and colleagues *(61)* investigated the relationship of vitamin C intake (evaluated using a food-frequency questionnaire) and knee pain among 324 participants (mostly males) over a 30-mo period in a longitudinal study of knee OA. Pain score was computed as an average of pain scores reported at all visits in their cross-sectional analysis. They found that individuals with low intakes of vitamin C (200 mg for men, 150 mg for women) had significantly more knee pain after adjusting for age, BMI, and energy intake. For example, among smokers in the lowest tertile for vitamin C intake, pain scores were 2.8 times greater than for all other men.

5.2.3. Clinical Trials (Table 3)

Benefit from vitamin E therapy has been suggested by several small studies of human OA *(62–65)*, of which the most rigorous was a company-sponsored, 6-wk, double-blind, placebo-controlled trial of 400 mg of α-tocophorol (vitamin E) in 56 patients with OA in Germany *(66)*. Vitamin E-treated patients experienced greater improvement in every efficacy measure, including pain at rest (69% better in vitamin E vs 34% better in placebo; $p < 0.05$), pain on movement (62% better on vitamin E vs 27% on placebo; $p < 0.01$), and use of analgesics (52% less on vitamin E vs 24% less on placebo; $p < 0.01$). The rapid response in symptoms observed in this study precludes a structural effect in this disorder and suggests that the beneficial effect might result from some metabolic action, such as inhibition of arachidonic acid metabolism.

Hill and Bird *(67)* conducted a 6-mo double-blind, placebo-controlled study of selenium–ACE, a proprietary nutritional supplement in the United Kingdom, among 30 patients with unspecified OA *(67)*. The active treatment contained 144 µg of selenium on average as well as unspecified quantities of vitamins A, C, and E. In fact, the placebo also contained 2.9 µg of selenium. Pain and stiffness scores remained similar for the two groups at all time points. The authors concluded that their data did not support efficacy for selenium–ACE in relieving symptoms of OA.

Wluka et al. *(68)* tested whether vitamin E (500 IU) affected cartilage volume loss in patients with knee OA in a 2-yr, double-blind, placebo-controlled trial among 136 patients with knee OA *(68)*. Tibial cartilage volume was measured by magnetic resonance imaging (MRI) at the beginning and end of the study. A total of 117 subjects completed the study. Loss of medial and lateral tibial cartilage, measured by MRI, was similar in subjects treated with vitamin E and placebo (e.g., mean loss in the medial compartment: 157 vs 187 m^3; $p = 0.5$). There also were no significant differences between the vitamin E- and placebo-treated groups in improvement of symptoms from baseline. They concluded that vitamin E does not appear to benefit cartilage volume loss or symptoms in people with OA.

5.3. Selenium and Iodine: Studies of Kashin–Beck Disease

Kashin–Beck disease is an osteoarthropathy of children and adolescents that occurs in geographic areas of China in which deficiencies of both selenium and iodine are endemic. Strong epidemiological evidence exists supporting the environmental nature of this disease *(69)*. Although the clinical and radiological characteristics of Kashin–Beck disease differ from OA, its existence raises the possibility that environmental factors also play a role in the occurrence of this disorder.

Table 3
Clinical Trials of Nutritional Products in Osteoarthritis

Author	*Compound tested*	*Comparator*	*Study n*	*Route of administration*	*Outcome*
Flynn *(74)*	Folic acid and cyanocobalamin	Placebo	30	Oral	No consistent difference between groups.
Hill *(67)*	Selenium ACE	Placebo	30	Oral	No effect observed.
Blankenhorn *(66)*	Vitamin E	Placebo	56	Oral	Favored vitamin E.
Reichelt *(96)*	Glucosamine	Placebo	155	Oral	Favored glucosamine.
Vajaradul *(98)*	Glucosamine	Placebo	54	Intra-articular	Favored glucosamine.
Drovanti *(93)*	Glucosamine	Placebo	80	Oral	Favored glucosamine.
Pujalte *(95)*	Glucosamine	Placebo	20	Oral	Favored glucosamine.
Muller *(105)*	Glucosamine	Obuprofen	200	Oral	Ibuprofen initially more efficacious; similar effects by week 4.
Vaz *(97)*	Glucosamine	Obuprofen	40	Oral	Ibuprofen initially more efficacious; similar effects after week 4.
L'hirondel *(101)*	Chondroitin	Placebo	125	Oral	Favored chondroitin.
Kerzberg *(102)*	Chondroitin	Placebo	17	Intra-muscular	Favored chondroitin.
Mazieres *(103)*	Chondroitin	Placebo	120	Oral	Favored chondroitin.
Conrozier *(122)*	Chondroitin	Placebo	129	Oral	Favored chondroitin.
Rovetta *(104)*	Chondroitin	Placebo	40	Intra-muscular	Favored chondroitin.
Cibere *(107)*	Glucosamine	Placebo	137	Oral	No effect observed.
Hughes *(109)*	Glucosamine	Placebo	80	Oral	No effect observed.
Michel *(114)*	Chrondroitin	Placebo	300	Oral	Favored chrondroitin.
Pavelka *(112)*	Glucosamine	Placebo	202	Oral	Favored glucosamine.
Reginster *(111)*	Glucosamine	Placebo	212	Oral	Favored glucosamine.
Morreale *(123)*	Chondroitin	Diclofenac	146	Oral	Favored chondroitin.

Selenium deficiency together with pro-oxidative products of organic matter (mainly fulvic acid) in drinking water and contamination of grain by fungi have been proposed as environmental causes for Kashin–Beck disease. However, the efficacy of selenium supplementation in preventing the disorder is controversial. Because selenium is an integral component of iodothyronine deiodinase as well as glutathione peroxidase, Moreno-Reyes et al. *(70)* studied iodine and selenium metabolism in 11 villages in Tibet in which Kashin–Beck disease was endemic and 1 village in which it was not. They found iodine deficiency to be the main determinant of Kashin–Beck disease in these villages, although it should be noted that selenium levels were very low in all the subgroups examined. In an accompanying editorial, Utiger inferred that Kashin–Beck disease probably results from a combination of deficiencies of both of these elements and speculated that growth plate cartilage is both dependent on locally produced triiodothyronine and sensitive to oxidative damage.

It should be noted that there is little, if any, evidence to suggest that Kashin–Beck disease has any similarities with OA. Furthermore, the single published clinical trial of supplemental selenium (selenium-ACE) in the treatment of symptoms associated with OA demonstrated no efficacy from this product *(67)*.

5.4. Studies of Vitamin D

In a separate investigation, we tested the association of vitamin D status on the incidence and progression of knee OA among the cohort of participants in the Framingham OA Cohort Study described earlier *(71)*. The methodology for this investigation was essentially identical, with the exception that the analysis was confined to the subset of individuals who both participated in the dietary assessment and provided serum for assay of 25-hydroxy vitamin D (25[OH]D; $n = 556$). Dietary intake of vitamin D and serum 25(OH)D levels were modestly correlated in this sample ($r = 0.24$) and, as in the previous study, were unrelated to incidence of OA. However, risk of progression increased threefold for participants in the middle and lower tertiles of both vitamin D intake (OR for lowest vs highest tertile = 4.0, 95% CI 1.4–11.6) and serum level (OR = 2.9, 95% CI 1.0–8.2). Low serum vitamin D level also predicted cartilage loss, as assessed by loss of joint space (OR = 2.3, 95% CI 0.9–5.5) and osteophyte growth (OR = 3.1, 95% CI 1.3–7.5). We concluded that low serum level and low intake of vitamin D were each associated with a highly significant increase in the risk of progression of knee OA.

Lane et al. *(72)* subsequently examined the relationship of serum 25- and 1,25-hydroxy vitamin D with the development of radiographical hip OA among Caucasian women older than age 65 yr who were participating in the Study of Osteoporotic Fractures. They measured serum vitamin D levels in 237 subjects randomly selected from 6051 women who had pelvic radiographs taken at both the baseline examination and after 8 yr of follow-up. Radiographs in this study were graded using a validated scoring system for hip OA based on individual radiographical features (osteophytes and joint space narrowing). The investigators analyzed the association of vitamin D levels (ranked in tertiles) with the occurrence of joint space narrowing, the development of osteophytosis, and changes in the mean joint space width and individual radiographical feature scores (treated as continuous variables) during the study period. Multivariate analyses were adjusted for age, clinic, weight at age 50 yr, and health status. They found a significantly increased risk for development of joint space narrowing among those in the lowest tertile for 25(OH)D (OR = 2.5, 95% CI 1.1–5.3) as

well as associations with continuous measures of progression (β = –0.1, 95% CI –0.2 to –0.02). An increased risk was also apparent for development of joint space narrowing among those in the middle tertile for 25-hydroxy vitamin D but did not reach statistical significance.

McLaughlin et al. *(55)* examined the effects of 25(OH)D and parathyroid hormone on the risk of progression among a cohort of 312 participants with symptomatic knee OA at a VA hospital. This study has been reported only in abstract form at this time. Progression was defined as worsening in joint space narrowing by at least one grade (range: 0–3) in either knee over a period of 30 mo or less. Serum levels were only available for 222 of the 312 participants, but there were no apparent significant differences between those for whom samples were or were not available (data are not reported in the abstract). They found no striking influence of these variables on OA progression in their analyses. Further data are awaited from this preliminary analysis. However, there are several important features of this study that might account for the discordance with the Framingham data. These relate to the characteristics of the VA hospital-based sample and the focus on joint space narrowing as the primary outcome measure. In particular, the levels of 25(OH)D were lower in the VA study such that the cut-point between the middle and upper tertiles (25 ng/mL) corresponded to the cut-point between the lower and middle tertiles for the Framingham cohort. Therefore, the VA sample may not have had sufficiently high levels of vitamin D to exert a detectable effect. Also, the classification of knee OA progression on the basis of joint space narrowing may have reduced differences toward the null if the biological benefit of vitamin D was exerted through bone rather than cartilage. For example, it is notable that in the Framingham cohort, the influence of serum 25(OH)D predicted osteophyte growth more strongly than cartilage loss.

Thus, two independent epidemiological studies demonstrated an inverse association of vitamin D status with risk for OA *(71,72)*. Both of these studies used a prospective design and included relatively robust measures of vitamin D. The studies also were concordant for the observation that individuals with the lowest risk exhibited 25(OH)D levels greater than 30 ng/mL. On the other hand, one VA hospital-based prospective study of knee OA progression found no such effects *(73)*. However, the individuals in this study tended to have lower levels of vitamin D. Furthermore, thus far, the analyses have been confined to prediction of joint space loss as the primary outcome.

5.5. Folic Acid and Cobalamin

Flynn et al. *(74)* performed a 2-mo double-blind, randomized, three-arm, crossover clinical trial of 6400 μg of folate vs 6400 μg of folate with 20 μg of cyanocobalamin or a placebo among 30 individuals with symptomatic hand OA. Participants were assessed for tender joints, grip strength, symptoms, and analgesic use. In their analyses, the authors stratified the participants according to their baseline grip strength. Ultimately, some benefits were found among some strata for certain measures of grip strength and tender joints favoring the folate/cyanocobalamin arm. However, few differences were noted regarding pain scores, global assessments, or analgesic use, suggesting that this intervention had limited, if any, efficacy.

Dietary intake of folate was also tested as a nonantioxidant control micronutrient in the Framingham Osteoarthritis Study. In this observational study, we found no convincing effect of this micronutrient on knee OA incidence or progression.

6. STUDIES OF OTHER NUTRIENTS IN OSTEOARTHRITIS

6.1. Glucosamine and Chondroitin Sulfate

6.1.1. Osteoarthritis Symptoms

The idea that administration of glucosamine or chondroitin sulfate might have therapeutic effects in treating OA by providing substrate for reparative processes in cartilage has existed since at least the 1960s. These compounds occur naturally in the body and may be involved in the repair and maintenance of normal cartilage. They have been used for many years in veterinary medicine for the symptomatic relief of arthritis. Recently, health and nutrition stores and numerous news shows and popular books have promoted the use of glucosamine and chondroitin sulfate for the treatment of arthritis. On the basis of anecdotal evidence, the products appear to be gaining popularity among consumers. Different formulations of glucosamine are available in nutrition stores, which sell these products as dietary supplements.

Although there has been continuing interest in these compounds, this appears to have been tempered by lack of a plausible mechanism to explain how they might achieve a therapeutic effect. In fact, recent laboratory studies have indicated that glucosamine is absorbed through the gastrointestinal tract *(75)* and then rapidly distributed throughout the body with selective uptake by articular cartilage *(76,77)*, where it stimulates both glycosaminoglycan and proteoglycan synthesis *(78–80)*. The biological fate of orally administered chondroitin sulfate is less clear, but some evidence exists to suggest that the compound may be absorbed following oral administration, possibly as a result of pinocytosis *(81)*. Chondroitin sulfate can cause an increase in RNA synthesis by chondrocytes *(82)*, which appears to correlate with an increase in the production of proteoglycans and collagens *(83–86)*. Additionally, there is evidence that chondroitin sulfate partially inhibits leukocyte elastase and, therefore, may reduce the degradation of cartilage collagen and proteoglycans, which is prominent in the OA process *(87–90)*.

Glucosamine and chondroitin sulfate have been the subject of numerous clinical trials in Europe and Asia, all of which had demonstrated favorable effects (until recently; refs. *91–105*) (*see* Table 3). We performed a meta-analysis and quality assessment of double-blind, placebo-controlled clinical trials of glucosamine and chondroitin compounds to evaluate their likely efficacy for OA *(106)*. Of the trials we studied, 13 met eligibility criteria. Quality scores were substantially lower for abstracts compared with manuscripts, with major deficiencies apparent in descriptions of randomization, blinding, and completion rates. Most, if not all, were sponsored by a manufacturer of the product. No negative studies were found. All studies were classified as positive and demonstrated large effects, with mean score reductions compared to placebo of 39.5 and 40.2% for glucosamine and chondroitin, respectively. We concluded that clinical trials of glucosamine and chondroitin showed benefits in the treatment of OA symptoms but provided insufficient information about study design and conduct to allow definitive evaluation.

However, the body of evidence concerning the efficacy of glucosamine was recently altered by the publication of four independently funded clinical trials, three of which had completely null results *(107–110)*. Rindone et al. *(110)* recruited 114 patients (mostly men) with knee OA from a VA medical center and randomized them into a 2-mo placebo-controlled trial of glucosamine sulfate, 500 mg tid. The participants had rather severe radiographical, OA and 56% were using additional analgesia drugs. Sixteen

participants were lost to follow-up or withdrew from the study. The investigators found no substantial or significant differences in outcomes between the two groups after either 30 or 60 d of treatment (e.g., mean change in knee pain on walking: glucosamine group = –1.4, placebo group = –1.5; $p = 0.77$, for the difference).

Hughes and Carr *(109)* performed a double-blind, placebo-controlled, randomized, controlled trial (RCT) of glucosamine sulfate (1.5 g/d) in patients recruited from a rheumatology clinic. Participants had relatively severe knee OA from both a symptomatic and radiographical perspective and were often using nonsteroidal anti-inflammtory drugs or other analgesics. Of those studied, 80 participants were randomized and 78 were included in the efficacy analysis. Adherence was greater than 80% in both treatment groups. The two groups were apparently similar regarding baseline demographical and OA-related variables, although these data were not provided in the paper. No significant differences were found between groups in any of the primary endpoints in this study. The two treatment groups also were similar for the secondary outcomes, with the exception of range of knee flexion, which showed a mean increase of 4° in the glucosamine group and a decrease of 9° in the placebo group ($p = 0.02$, for the difference). The authors were skeptical about the credibility of this finding because the magnitude of the difference is less than the minimal detection limit of a goniometer.

Cibere et al. *(107)* presented preliminary results of an innovative glucosamine withdrawal trial in people with knee OA who were already using the product and who reported at least moderate benefit. Enrollees were randomly assigned to continue taking glucosamine sulfate or to placebo. The intervention continued for 24 wk or until the subject met predefined criteria for a disease flare. A total of 137 participants were enrolled. Prior use of glucosamine was variable regarding dose and (presumably) manufacturer. Rates of disease flare were essentially identical (43 vs 42%) in the two treatment arms, although a small, nonsignificant reduction in risk was observed after adjustment for gender and radiographical severity (hazard ratio = 0.8, 95% CI = 0.5–1.4). No substantial differences were noted between treatment groups regarding the secondary outcomes, including Western Ontario and McMaster Universities scores and level of analgesic use. One of the problems in interpreting the results of this study is that if the duration of effectiveness of glucosamine is prolonged (as claimed), then the period of follow-up in may have been insufficient. Also, the heterogeneity of glucosamine products on the market could have biased any differences to the null.

6.1.2. Studies of Osteoarthritis Process Modification

Rotta Pharmaceuticals sponsored two large, multicenter RCTs to examine the possibility that glucosamine might reduce rate of loss of articular cartilage *(111,112)*. These trials enrolled approx 200 outpatients with primary knee OA into 3-yr RCTs comparing Rotta glucosamine sulfate (1.5 g taken once daily) with placebo. The primary outcome in each trial was based on joint space measurements obtained from standing anteroposterior knee radiographs, the recommended radiographical approach at that time. Both trials showed quantitatively similar benefits in the glucosamine treatment armsregarding the rate of loss of joint-space width and symptoms. Unfortunately, the approach that was used to estimate width of joint space in these RCTs has proved to be problematic, although it was the recommended technique at the inception of the trials *(113)*. Precise measurement of this variable is contingent on highly reproducible radioanatomic positioning of the joint and may be biased by the presence of pain.

Interestingly, similar influences of chondroitin sulfate on radiographical progression of knee OA were also reported in abstract form based on results of a 2-yr RCT, placebo-controlled study of 100 participants *(114)*.

7. OTHER NUTRITIONAL PRODUCTS

An increasing number of nutritional remedies have apparently been promulgated for purported benefits in arthritis. Several of these have been tested in controlled clinical trials. For example, four trials of avocado/soybean unsaponifiables were pooled in a meta-analysis that displayed guardedly positive results *(115)*. Trials of *S*-adenosylmethionine also had apparently positive results, although they were somewhat limited by adverse effects and high drop-out rates *(116–120)*. An 32-wk study of ayurdevic remedy, prepared from certain herbs and roots, was also tested in an industry-sponsored, placebo-controlled clinical trial; however, this was presented only as an abstract *(121)*.

8. CONCLUSIONS AND RECOMMENDATIONS

The idea that nutritional factors might influence the occurrence or course of OA is relatively new to the scientific community (although not to the general public). As a result, research in this field is at an early stage, and few definitive conclusions can be reached. Nevertheless, it is clear that there are many plausible mechanisms through which various nutritional factors might influence the occurrence and course of this major public health problem. Preliminary findings suggest a possible role of vitamin C in reducing OA pain and pathological progression. Apparent benefits from vitamin D were observed for two different joint sites in two large epidemiological studies, but benefits were absent in a third study. The interpretation of clinical trial data regarding the effects of glucosamine and chondroitin sulfate on OA symptoms was recently clouded by the publication of several independent trials that had negative results.

What might be reasonable nutritional advice for an individual with OA, given the incomplete state of current knowledge? From a pragmatic standpoint, the data suggest interventions that are largely concordant with general advice about healthy eating, particularly increasing consumption of fresh fruit and vegetables and optimizing vitamin D status. It is notable in the Framingham Osteoarthritis Study that an individual could move out of the high-risk tertile for vitamin C intake by increasing consumption by as little as an orange per day. For vitamin D, an intake of 400 IU/d or serum levels of greater than approx 30 ng/mL appear to confer maximum benefit. Vitamin D is derived from a number of sources, and there is a small risk of toxicity for individuals consuming large daily doses. Supplementation with 400–800 IU/d appears to be safe. For people in whom there are concerns about adequate supplementation, or toxicity, we recommend measurement of serum 25(OH)D levels.

Regarding nutritional products, there appear to be no compelling reasons to discourage the use of glucosamine and chondroitin sulfate. These products are substantially safer than many drugs prescribed in the treatment of OA symptoms; however, they are often expensive, lack pharmaceutical-level manufacturing standard controls, and may not work.

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17 Calcium Requirements During Treatment of Osteoporosis in Women

Richard Eastell

KEY POINTS

- The major studies of osteoporosis therapies have ensured that adequate calcium and vitamin D was available, usually administered as supplements.
- Many patients with osteoporosis have inadequate dietary intake of calcium and vitamin D insufficiency.
- The calcium requirement is increased during treatment with anti-resorptive therapy for osteoporosis.
- Vitamin D deficiency impairs the response to anti-resorptive therapy for osteoporosis.
- Calcium supplementation results in a decrease in bone resorption, and this is the likely mechanism for the decrease in fracture risk.
- I recommend that about 1000 mg of calcium and 800 IU of vitamin D be given along with anti-resorptive therapy for osteoporosis.

1. INTRODUCTION

Almost all of the phase III clinical trials for the treatment of postmenopausal osteoporosis included a calcium supplement, and most included vitamin D *(1)*. However, when these treatments were approved by the regulatory authorities, the only recommendation made was that the diet should contain sufficient calcium and vitamin D. This chapter considers whether the calcium and vitamin D is simply a placebo in these studies, and whether women with osteoporosis commonly attain adequate intakes of calcium and vitamin D without supplementation.

1.1. How Much Calcium and Vitamin D Was Used in the Key Phase III Clinical Trials for Osteoporosis?

There have been many clinical trials of drugs for postmenopausal osteoporosis. A summary of the major clinical trials is included in Table 1. The usual doses of calcium and vitamin D supplementation were between 500 and 1000 mg/d and 250 and 600 IU/d, respectively. These amounts of vitamin D would be expected to increase serum 25-hydroxyvitamin D [25-(OH)D] by between 6 and 15 nmol/L *(2)*.

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Table 1
The Level of Calcium and Vitamin D Supplementation in Several Large Clinical Trials of Postmenopausal Osteoporosis

Name of trial	*Antiresorptive drug*	*Calcium (mg/d)*	*Vitamin D, IU/day*
FIT 1 and 2 *(3,24)*	Alendronate	500[a]	250
VERT MN and NA *(4,25)*	Risedronate	1000	Up to 500[b]
PROOF *(26)*	Calcitonin	1000	400
MORE *(27)*	Raloxifene	500	Up to 600

[a] If dietary calcium is less than 1000 mg/d (in 82% of subjects in FIT 1).
[b] If 25(OH)D is less than 40 nmol/L (in 36% of subjects in VERT-MN).
FIT, Fracture Intervention Trial; VERT, Vertebral Efficacy with Risedronate Therapy; PROOF, Prevent Recurrence of Osteoporotic Fractures; MORE, Multiple Outcomes of Raloxifene Evaluation; MN, multinational; NA, North America.

1.2. Are These Levels Reached in the Habitual Diet?

The recommendation in the Fracture Intervention Trial Study was that a food-frequency questionnaire be used to evaluate dietary calcium *(3)*. If the dietary calcium was below 1000 mg/d, then the patient should be given 500 mg of calcium per day. About 82% of women with osteoporosis failed to achieve this level.

In the Vertebral Efficacy with Risedronate Therapy (VERT) Studies, the recommendation was to measure 25(OH)D and to supplement with vitamin D (up to 500 IU/d) if the value of 25(OH)D was below 40 nmol/L. About 36% of patients in the multinational VERT Study required supplementation *(4)*. This threshold is quite low; it is becoming accepted that a threshold of 80 nmol/L is desirable to prevent the biochemical changes of subclinical osteomalacia (high parathyroid hormone [PTH] and bone turnover markers) *(2)*. Thus, to reproduce the conditions under which these trials were conducted, it is likely that most people receiving treatment for osteoporosis need supplementation with calcium and vitamin D.

2. RATIONALE FOR SUPPLEMENTATION

2.1. Positive Calcium Balance With Treatment

There is a positive calcium balance after starting therapy with anti-resorptive therapy. Thus, the total body bone mineral content increases by 1% at 1 yr after treatment with alendronate plus calcium (500 mg/d) *(5)*. This is equivalent to a positive balance of about 20 mg/d. In women given 500 mg/d of calcium alone, there was 0.5% decrease in total body mineral content; this is equivalent to a negative calcium balance of about 10 mg/d, so the extra requirement resulting from administration of 10 mg/d of alendronate is 30 mg/d. This requirement can be fulfilled by an adaptation of the body by an increase in the fraction of dietary calcium absorbed *(6)*. However, this mechanism is less effective in the elderly. It can be achieved by the calcium supplement: if about 10% is absorbed, then an extra 50 mg/d of calcium is available to support the positive calcium balance.

2.2. Secondary Hyperparathyroidism

The period of positive calcium balance represents a decrease in net bone resorption, which in turn results in a tendency for serum calcium to decrease. The parathyroid glands compensate for this decrease by increasing serum PTH and, consequently,

increasing the serum calcitriol level *(7)*. It is unclear whether the effect of the increase in PTH is beneficial or not. The increase in PTH may be attenuated, but not prevented, by the coadministration of supplemental calcium.

2.3. Vitamin D Deficiency Prevents Antiresorptive Effect and Impairs Muscle Strength

Koster et al. *(8)* recruited 28 osteopenic patients with vitamin D deficiency [25(OH)D <40 nmol/L) and compared their bone mineral density (BMD) response to 1 yr of cyclical etidronate with a control group that had normal 25(OH)D. The control group showed an increase in BMD of 2 to 6% in response to cyclic etidronate, depending on the site of measurement. The vitamin D-deficient group had little or no improvement in the BMD and further deterioration of the vitamin D deficiency, as indicated by a decrease in serum calcium and an increase in PTH and osteocalcin. It is likely that the vitamin D deficiency prevented the increase in calcitriol. Thus, there would be no increase in calcium absorption in response to the etidronate therapy.

There is some evidence that vitamin D therapy might reduce the risk of falls in postmenopausal women. Pfeifer et al. *(9)* reported that 148 women with low 25(OH)D (<50 nmol/L) were given a calcium supplement (1000 mg/d) and randomized to vitamin D (800 IU/d) or placebo for 1 yr. Calcium supplementation with or without vitamin D supplementation resulted in a significant decrease in serum PTH; however, the vitamin D supplementation resulted in a further decrease in serum PTH. The vitamin D also resulted in decreases in both body sway and the average number of falls per subject. Bischoff et al. *(10)* reported that 122 elderly women were given a calcium supplement (1200 mg/d) and randomized to vitamin D (800 IU/d) or placebo for 3 mo. The vitamin D resulted in a 49% decrease in the average number of falls per subject and an improvement in musculoskeletal function. Both studies assessed the number of falls and not the number of subjects who fell; this is a limitation of the studies. Nonetheless, this reduction in the risk of falling could translate into a decrease in the risk of fracture.

3. EFFECTS OF CALCIUM SUPPLEMENTATION ON BONE TURNOVER AND BMD

The effect of calcium supplementation depends on the dose and the population in which it is studied. There is a positive effect on BMD in women who are more than 6 yr after menopause, and the effect is more clearly seen in those women taking a diet that is low in calcium (<400 mg/d) *(11)*. The effect is present at the usual sites measured—the distal forearm, spine, proximal femur, and total body. The effect is maximal in the first year and is reversible (once the supplement is stopped, the effect resolves).

The effect of calcium supplementation is dose-dependent, and it depends on the time of day the dose is administered. If the calcium is administered in the evening, then the normal nocturnal increase in bone resorption is prevented and, therefore, the 24-h resorption rate is reduced more than if the supplement is administered in the morning *(12)*.

Nieves et al. *(13)* determined that hormone-replacement therapy and calcitonin trials that included a calcium supplement resulted in an extra 1 to –2% gain in BMD when compared to those trials without a calcium supplement.

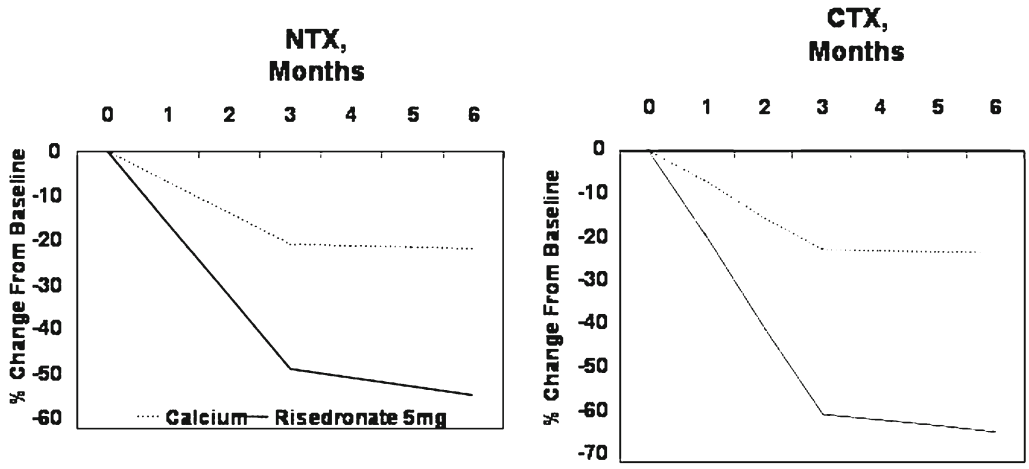

Fig. 1. The effect of calcium supplementation in postmenopausal osteoporosis. The lines show the median change in the bone resorption markers urinary NTX and CTX in 693 women with osteoporosis treated with calcium (with or without vitamin D) alone in broken lines or with risedronate 5 mg/d (solid lines). (Data from Eastell et al. *[15]*).

3.1. Effect on Bone Turnover

3.1.1. Magnitude

Bone resorption can be assessed by measuring the degradation products of type I collagen; these include deoxypyridinolone and the telopeptides of type I collagen, NTX and CTX. The effect of calcium (and vitamin D) is to decrease bone turnover markers. The biochemical markers of bone resorption decrease within 4 h of administration of the first dose of calcium to the maximum level of suppression *(14)*. The markers remain suppressed for as long as the calcium is administered. The size of the effect depends on the dose and on the nature of the bone resorption marker. For a marker such as urinary NTX, the decrease in women with postmenopausal osteoporosis averages about 20% (Fig. 1; ref. *15*).

Calcium plus risedronate results in a median decrease in urinary NTX of 51% (Fig. 1; ref. *16*). However, if women are already taking calcium supplements, then the mean decrease in urinary NTX is only 37% (unpublished data, Improving Measures of Persistance on "Actonal" Treatment [IMPACT] study).

3.2. Mechanism for the Reduction in Bone Turnover

The decrease in bone turnover with calcium supplementation is mainly a consequence of the decrease in serum PTH. Thus, within 1 h of administration of calcium supplements, the serum PTH begins to decrease and is maximal at about 2 h; this change correlates with the decrease in bone resorption markers at 4 h *(17)*. Ledger et al. *(18)* conducted a calcium infusion study in older women over a period of 24 h and showed that the serum PTH and the urinary NTX excretion decreased to the levels found in healthy women, indicating that the increase in PTH with aging is an important cause of the age-related increase in bone resorption.

There could be other mechanisms for the suppression of bone resorption markers. It may be that osteoclasts have calcium-sensing receptors and that they respond to the

very small increase in serum-ionized calcium after administration of a calcium supplement. Another alternative is that the intestine secretes a factor in response to calcium supplementation. One such factor might be an increase in gastrin that would subsequently stimulate the secretion of calcitonin.

4. EFFECTS OF CALCIUM SUPPLEMENTATION ON FRACTURE RISK REDUCTION

4.1. Relationship Between the Change in Bone Turnover and Fracture Risk Reduction

The decrease in the bone resorption markers have explained much of the antifracture effect of antiresorptive therapy, even more so than the increase in BMD. In one study, the decrease in NTX and CTX appeared to explain up to 66% of the reduction in vertebral fractures and up to 77% in nonvertebral fractures *(15)*. The same holds true for the effect of calcium supplementation on vertebral fractures. Thus, the relationship between the change in bone resorption markers and vertebral fracture risk reduction in the group given calcium alone was significant, indicating that with calcium, the greater the reduction in bone resorption markers in an individual, the greater the reduction in vertebral fracture risk (Fig. 2).

4.2. Clinical Trials of Fracture Risk Reduction

Shea et al. *(19)* performed a meta-analysis of the studies with calcium alone on BMD and on fracture. They believed that up to 2000, there were 15 trials of sufficient quality to include in their analysis. They reported that at 2 yr, calcium supplementation had an effect on the BMD of the total body (2.1%), lumbar spine (1.6%), proximal femur (1.7%), and distal radius (1.9%). The relative risk of fractures of the vertebrae was 0.77 (95% confidence interval [CI] 0.54–1.09), and for nonvertebral fractures, the relative risk was 0.86 (95% CI 0.43–1.72). However, this analysis omits two of the key studies. These studies included vitamin D and, therefore, were considered in a separate analysis reported by Papadimitropoulos et al. *(20)*. They reported that for nonvertebral fractures, the relative risk for fracture was 0.78 (95% CI 0.55–1.09). It is surprising that the latter result did not reach conventional levels of significance, because it included three trials, two of which had a significant result *(21,22)*. The main influence on the analysis was the trial of Lips *(23)*, which did not include a calcium supplement and used a relatively low dose of vitamin D (400 IU/d as compared to 700 and 800 IU/d for the other two studies).

On balance, it is likely that calcium and vitamin D, when given in adequate doses (e.g., 1000 mg/d of calcium and 800 IU/d of vitamin D) reduce the risk of vertebral and nonvertebral fractures in postmenopausal women. However, this effect is not as great as the effects of treatments such as risedronate, alendronate, or Raloxifene.

5. CONCLUSIONS AND RECOMMENDATIONS

Almost all of the clinical trials of treatments for osteoporosis included calcium and/or vitamin D as supplements. These supplements are more effective than placebo and act in their own right to reduce the risk of fracture. It is likely, although not proven, that they add to the antifracture effects of the antiresorptive drugs with which they are

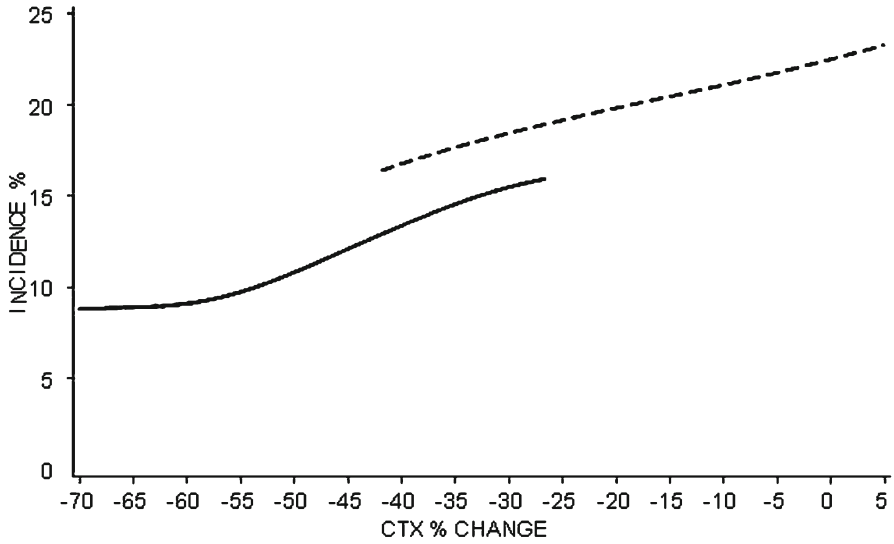

Fig. 2. The relationship between the change in bone resorption marker (CTX) and the risk of new vertebral fracture. There was a significant relationship overall between the decrease in bone resorption and the decrease in risk of vertebral fracture, and this was true for the calcium group alone (broken line). Solid line represents the calcium plus risedronate group.

administered. It would appear that the supplemental dose that would help reduce fractures and strengthen muscle would be about 1000 mg of calcium and 800 IU of vitamin D per day.

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18 Osteoporosis

Protein, Minerals, Vitamins, and Other Micronutrients

Robert P. Heaney

KEY POINTS

- Bone health requires total nutrition, because the integrity of bone tissue depends on the integrity of its cells, which (similarly to most other tissues) need a broad array of macro- and micronutrients. Additionally, calcium and protein play key roles, because the bulk of the bony material is made up of these substances.
- Bone turns over relatively slowly. Thus, the effects of inadequate nutrition on bone often are delayed, and the structural properties of bone tend to reflect past nutrition more than current intakes.
- Calcium is a threshold nutrient. The minimum daily requirement is the intake at which bony response plateaus. To reach this threshold, calcium intake should be 1500 mg/d both during growth and after age 50. With lifelong calcium intakes in this range, risk of osteoporotic hip and other nonspine fractures can be reduced by 30 to 50%.
- Vitamin D is predominantly produced in the skin. Recommended daily oral intakes are sufficient only to prevent the most extreme bony manifestations of vitamin D deficiency. Optimal vitamin D status is ensured by serum 25-hydroxyvitamin D [25(OH)D] values of 80 nmol/L (32 ng/mL) or more. Lower values are associated with impaired calcium absorption and increased osteoporotic fracture risk. Daily use of vitamin D may be as high as 4000 IU (100 μg). For most elderly individuals, a daily oral dose of 1000 IU or higher is necessary to sustain adequate serum 25(OH)D concentrations.
- Protein, once thought to be potentially harmful to bone when ingested in large quantities, is now best understood as complementary to calcium. Together, the two nutrients provide the bulk constituents of bony material; to achieve the full benefit of either, the intake of the other must be adequate as well. Protein intakes that optimize bony response are uncertain but, from available data, appear to be above the current Recommended Daily Allowance (RDA; 0.8 g/kg of body weight).
- Although typical magnesium intakes are below the RDA (310 and 400 mg/d for women and men, respectively), there appear to be no skeletal consequences of the shortfall. Supplemental magnesium does not improve calcium absorption in individuals consuming typical diets and has no effect on calcium balance.

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- Vitamin K, zinc, manganese, and copper are involved in various aspects of bone matrix formation, but it is not known whether deficiency of any of these contributes to the development or severity of typical osteoporosis.
- Recovery from hip fracture can be substantially improved with aggressive attention to the nutritional status of patients with hip fractures, with special emphasis on repairing the protein malnutrition that is common in such patients.

1. INTRODUCTION

1.1. Nutrition in the Osteoporotic Fracture Context

Osteoporosis currently is defined as a condition of skeletal fragility resulting from decreased bone mass and microarchitectural deterioration of bone tissue, with consequent increased risk of fracture. The condition is multifactorial in pathogenesis. Nutrition affects bone health in two distinct ways. First, bone tissue deposition, maintenance, and repair are the result of cellular processes, which are as dependent on nutrition, as are the corresponding processes of any other tissue. The production of bone matrix, for example, requires the synthesis and posttranslational modification of collagen and an array of other proteins. Nutrients involved in these cellular activities include not only the amino acid building blocks of the protein itself but vitamins C, D, and K and the minerals phosphorus, copper, manganese, and zinc. Additionally, the regulation of calcium homeostasis through modulation of bone resorption requires normal magnesium nutrition. Second, the skeleton serves as a very large nutrient reserve for two minerals, calcium and phosphorus, and the size of that reserve (ultimately equivalent to the strength of the skeletal structures) is dependent, in part, on the daily balance between absorbed intake and excretory loss of these two minerals.

Similar to most engineering structures, strength in bone is dependent not only on its massiveness but also on the arrangement of its material in space and on the intrinsic strength of its component material (particularly in bone, because over long periods of use, that strength is influenced by the accumulation of unrepaired fatigue damage as well as by the prevalence and location of remodeling loci, which are locally weak until fully repaired). All three factors play a role in most low-trauma fractures, and it often is not possible to say which may be the most important in any given case.

Bone mass and density are influenced by many factors in industrialized nations. Physical activity, gonadal hormones, and nutrition are the three factors that are most commonly found to limit achieving the bone mass that is genetically possible. In adults of these nations, calcium and vitamin D are the nutrients most likely to be in short supply. Specifically, calcium intake may be inadequate because it is low; however, even when statistically "normal," it still may be inadequate because of subnormal absorption *(1)* or greater than normal excretory loss *(2,3)*.

Because calcium and vitamin D are the nutrients most likely to be limiting in the industrialized nations, much of the following discussion focuses on these two nutrients. It is necessary to stress at the onset that both function mainly as nutrients—not as drugs. Therefore, their beneficial effects are confined to individuals whose intake of either is insufficient. Also, calcium is not an isolated nutrient; it occurs in foods along with other nutrients, and it has been shown that diets low in calcium also tend to be nutritionally poor in other respects as well *(4)*. Thus, although it is necessary to deal with nutrients one by one in an analysis such as this chapter, it is useful to bear in mind that the disorders in our patients are likely to be more complex.

2. CALCIUM

The primitive function of the skeleton is to serve as a source and as a sink for calcium and phosphorus, that is, as a reserve to offset shortages and as a place for safely storing dietary surpluses, at least after periods of depletion. For example, we see this reserve feature of skeletal function expressed in laboratory animals such as cats, rats, and dogs, which, when placed on low-calcium intakes, show reduced bone mass as needed to maintain near constancy of calcium levels in the extracellular fluid *(5)*. This activity is mediated by parathyroid hormone (PTH *[6]*) and involves actual bone destruction, not leaching of calcium from bone.

By nature, reserves are designed to tide organisms over external shortages. When intake is inadequate, the reserve is the first component of the body content to be depleted. With most nutrients, this depletion of the reserve has no detectable impact on the health or function of the organism. Only after the reserve is exhausted and the metabolic pool begins to be depleted does clinical disease express itself. For some nutrients (e.g., vitamin A, energy), the reserve can be quite large, and the latent period may last many months. However, for others (e.g., the water-soluble vitamins), the reserve may be very small, and detectable dysfunction develops quickly when intake drops.

Calcium is a unique nutrient in that over the course of evolution of the higher vertebrates, the calcium reserve acquired a second role, namely, internal stiffening and rigidity; today, this is the most apparent feature of the skeleton. Calcium is the only nutrient with a reserve that possesses such a major—if secondary—function, and the size of the reserve is unusually large relative to the cellular and extracellular metabolic pools of calcium. As a result, dietary insufficiency virtually never directly impairs tissue functions that are dependent on calcium, at least not in ways we now recognize. However, because bone strength is a function of bone mass, it inexorably follows that any decrease whatsoever in the size of the calcium reserve (any decrease in bone mass) will produce a corresponding decrease in bone strength. We literally walk on our calcium reserve. It is this unique feature of calcium nutriture that is the basis for the link between calcium intake and bone status.

2.1. Ascertaining the Requirement for Calcium

Calcium functions as a threshold nutrient, similarly to iron. This means that below some critical value, the nutrient effect (bone mass for calcium and hemoglobin mass for iron) is limited by available supplies, whereas above that value (i.e., the "threshold,"), no further benefit for that particular function accrues from additional increases in intake. This biphasic relationship is illustrated in Fig. 1, in which the intake–effect relationship is depicted first schematically (Fig. 1A) and then as exemplified by bone calcium data derived from a growing animal model (Fig. 1B). Figure 1B directly expresses the effect of the nutrient as the amount of bone calcium an animal is able to accumulate from any given intake. The minimum requirement can be defined as the intake at which the curve first becomes flat.

However, the same basic relationship holds throughout life, even when bone may be undergoing some degree of involution. The threshold concept is generalized to all life situations in Fig. 2A, which schematically demonstrates what the intake/retention curves look like during growth, maturity, and involution. In brief, the plateau occurs at

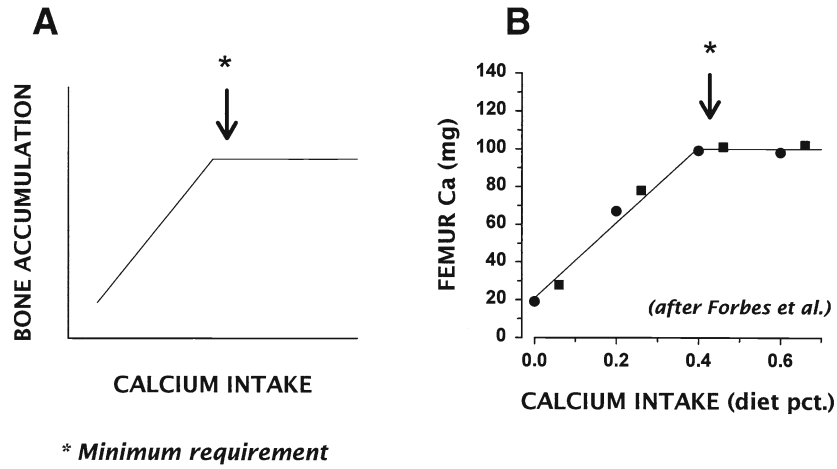

Fig. 1. Threshold behavior of calcium intake. (A) Theoretical relationship of bone accumulation to intake. Below a certain value (the threshold), bone accumulation is a linear function of intake (the ascending line); in other words, the amount of bone that can be accumulated is limited by the amount of calcium ingested. Above the threshold (the horizontal line), bone accumulation is limited by other factors and is no longer related to changes in calcium intake. (B) Actual data from two experiments in growing rats, showing how bone accumulation does, in fact, exhibit a threshold pattern. (Redrawn from data in; ref. *7*; Copyright Robert P. Heaney, 1992. Reproduced with permission.)

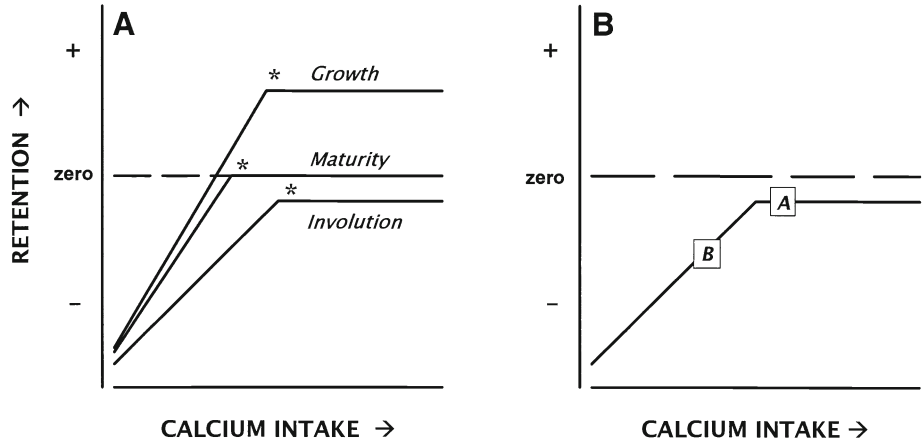

Fig. 2. (A) Schematic calcium intake and retention curves for three life stages. Retention is greater than zero during growth, zero at maturity, and may be negative during involution. (B) The involution curve only. Point B designates an intake below the maximal calcium retention threshold, and Point A represents an intake above the threshold. (Copyright Robert P. Heaney, 1998. Reproduced with permission.)

a positive value for retention during growth, at zero retention in the mature individual, and, sometimes, at a negative value in the elderly. (Available evidence suggests that the plateau during involution is negative in the first 3 to 5 yr after menopause, rises to zero for the next 10 to 15 yr, and then becomes increasingly negative in the elderly.) At all

Table 1
Various Estimates of the Calcium Requirement in Women

Age	*1989 RDA*	*NIH*[a]	*1997 DRI (AI)*[b]
1–5	800	800	500/800
6–10	800	800–1200	800/1300
11–24	1200	1200–1500	1300/1000
Pregnancy/lactation	1200	1200–1500	1000
24–50/65	800	1000	1000/1200
65+	800	1500	1200

Abbreviations: RDA, recommended daily allowance; NIH, National Institutes of Health; DRI (AI), daily recommended intake (adequate intake).

[a]Recommendations for women as proposed by the Consensus Development Conference on Optimal Calcium Intake *(8)*.

[b]"AI" refers to "adequate intake" *(9)*, a value that, in this context, is equivalent to an average requirement. The corresponding RDA could be 20–30% higher (i.e., 1000 in children, 1600 in adolescents, 1200 in adults to age 50 yr, 1200 during pregnancy and lactation, and 1450 in those over age 50 yr). The presence of two values reflects the fact that the age categories for the DRIs overlapped those of the NIH.

life stages, the best representation of the minimum requirement is the intake value just at or above the effect threshold shown in Figs. 1 and 2.

In Fig. 2B, which shows only the involutional curve, there are two points located along the curve, one below (*B*) and one above (*A*) the threshold. At point *A*, calcium retention is negative for reasons intrinsic to the skeleton, whereas at *B*, involutional effects are compounded by inadequate intake, which makes the balance more negative than it needs to be. Point *B* (or lower) is probably where most elderly adults in the industrialized nations would be situated today (*see* below). The goal of calcium nutrition in this life stage is to move to point *A* and thereby ensure that insufficient calcium intake is not aggravating any underlying bone loss.

There has been much uncertainty and confusion in recent years about what that intake may be for various ages and physiological states. With the 1994 Consensus Development Conference on Optimal Calcium Intake *(8)* and the dietary reference intakes (DRIs) released by the Food and Nutrition Board of the Institute of Medicine *(9)*, the bulk of that confusion has been resolved. The evidence for the intakes recommended by these expert panels is summarized both in their respective reports and in recent reviews of the relationship between nutrition and osteoporosis *(10,11)*, and only the highlights are mentioned in this chapter.

However, it is worth noting that the recommendations of both panels, although expressed in quantitative terms, are basically qualitative and can be summarized as follows: Contemporary calcium intakes in the United States, by both men and women, are too low for optimal bone health. The most persuasive of the evidence leading to this conclusion came in the form of several randomized controlled trials showing reduction in both age-related bone loss and in fractures following augmentation of prevailing calcium intakes *(12–19)*. For technical reasons relating to bone remodeling biology *(20)*, randomized controlled trials are not well-suited to dose ranging. Therefore, although the evidence was persuasive that prevailing intakes were too low, recommended levels in several cases involved ranges and were clearly prudential judgments centered of necessity on intakes employed in the trials concerned. The consensus panel's recommendations, the DRIs of the Institute of Medicine, and the corresponding 1989 RDAs *(21)* are set forth in Table 1.

2.2. Primary Prevention: The Acquisition of Genetically Programmed Bone Mass

At birth, the human skeleton contains approx 25 to 30 g of calcium; at maturity in women, it contains 1000 to 1200 g of calcium. This difference must be provided via the diet. Furthermore, unlike other structural nutrients such as protein, the amount of calcium retained is always substantially less than the amount ingested. This is both because absorption efficiency is relatively low even during growth and because calcium is lost daily through shed skin, nails, hair, and sweat as well as in urine and unreclaimed digestive secretions. With the exception of during infancy and the pubertal growth spurt, when retention may be as much as 25 to 30% of intake, only about 4 to 8% of ingested calcium is retained each day during most of the growth period. This inefficient retention is not so much because ability to build bone is limited but because the primitive calcium intake to which our physiologies are adapted was high. An intestinal absorptive barrier is a protection against calcium surfeit, and inefficient cutaneous and renal retention reflects primitive environmental abundance.

When ingested calcium is less than optimal, the balance between bone formation and resorption, normally positive during growth, falls toward zero. This occurs because PTH augments bone resorption at the endosteal–trabecular surface of growing bones to sustain the level of ionized calcium in the extracellular fluid, which has to meet the demand of ongoing mineralization at the periosteum and growth plates. When this demand exceeds the amount of calcium absorbed from the diet and released from growth-related bone modeling, more PTH is secreted, and resorption increases further until the balance becomes zero or negative. However, growth in bone size continues, and a limited quantity of mineral must now be redistributed over an expanding structural volume.

Net bone accumulation is greater as calcium intake increases, but only to the point where endosteal–trabecular resorption results solely from the genetic program governing the shaping of bone during growth and is not being driven by body needs for calcium. Above that level, as depicted in the data of Fig. 1B, further increases in calcium intake produce no further bony accumulation. The intake required to achieve the full genetic program and thus to assure peak bone mass is the intake that corresponds to the beginning of the plateau region in Fig. 1. This value is different for different stages of growth, partly because growth rates are not constant and also because as body size increases, obligatory calcium losses through skin and excreta increase as well.

By combining the many published reports of calcium balance studies during growth, it is possible to demonstrate in humans the pattern of plateau behavior found in laboratory animals and then to estimate, from the aggregated data, the intake values that correspond to the threshold *(22,23)*. Figure 3 represents one example of the relationship between intake and retention, combining the results of many published studies of calcium balance derived from a subset of the adolescents whose balances were assembled by Matkovic *(24)*. Figure 3 clearly shows the plateau type of behavior that both animal studies and theoretical considerations predict. It also shows that at intakes less than the plateau threshold, daily storage is less than optimal (i.e., accumulation of bone is limited by intake). Therefore, any such limiting of intake must be considered inadequate.

As demonstrated in Fig. 3, the daily threshold value for adolescents is about 1500 to 1600 mg. The best available estimates for the value of this daily threshold from balance studies performed at other stages of growth are 1400 mg in children and 1000 mg in young adults to age 30 yr (*see* Subheading 2.3.). The dual intake balance studies of

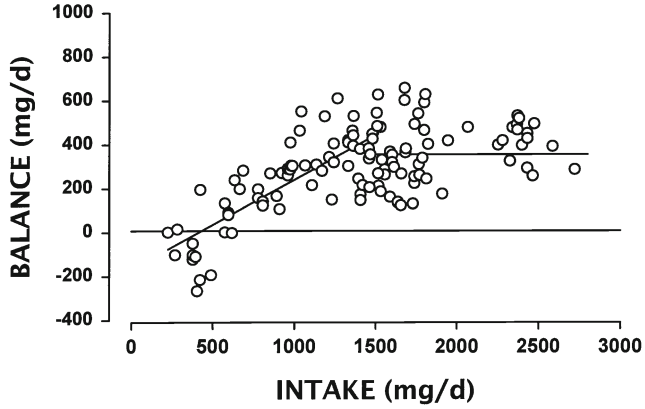

Fig. 3. The relationship of calcium intake, on the horizontal axis, to calcium retention (balance), on the vertical axis, for a subset of the adolescents described by Matkovic and Heaney *(22)*. Note that despite the "noisiness" that is inevitable in measurements of balance in humans, there is clear evidence of an intake plateau, as observed in the animal experiments of Fig. 1. Note also that, for this age, the threshold of the plateau occurs at about 1500 mg of Ca/d. (Copyright Robert P. Heaney, 1992. Reproduced with permission.)

Jackman et al. *(23)* found that 1300 mg/d in adolescents was the lowest intake that was consistent with their model for the retention plateau.

These values, based on analysis of balance data, are buttressed by several randomized controlled trials of calcium supplementation in children and adolescents *(15,16,25,26)* and by a longitudinal observational study in young adults *(27)*. The controlled trials demonstrated that bone gain during growth was greater when intake was elevated above the 1989 RDA (800 mg/d). Some of the gain that was seen within the first 6 to 12 mo of augmented calcium intake represented a phenomenon known as the remodeling transient *(20)*, which, although it conferred improved bone strength in its own right, could not be used to estimate the requirement because it mainly reflected the transition between two bone remodeling steady states. Nevertheless, computer modeling of the transient in these trials indicated that not all of the additional gain could be explained solely as a transient. Therefore, these data suggest that the 1989 RDAs lie on the ascending portion of the threshold curves of Fig. 2 rather than on the plateau, where they should lie. This explains the upward revisions reflected in the recommendations of the National Institutes of Health (NIH) Optimal Intake Panel and of the Food and Nutrition Board.

Despite the evidence just cited, pointing to relatively high intake thresholds for maximal calcium retention during growth, epidemiological data indicate that differences in peak bone mass related to habitual calcium intake are less than might be anticipated. This discrepancy suggests that individuals with habitually lower intakes may catch up to some extent. This seems to be the case in the controlled trial of Matkovic et al. *(28)*, in which not only did catch-up seem to occur in the unsupplemented adolescent girls, but the calcium benefit was most apparent in the tallest subjects (i.e., those with the greatest growth need for calcium).

The longitudinal study of young adults *(27)* showed prospectively that bone augmentation continues into the third decade of life. Bone mass gains in this study ranged

from 0.5% per year for the forearm to 1.25% per year for total body bone mineral. The single most important correlate of the rate of bone accumulation was calcium intake. Although this study lacked the inferential power of a randomized, controlled trial (RCT), it had an advantage over such trials in that it studied individuals on their self-selected intakes (i.e., at a steady state for bone remodeling) and thus avoided the confounding effect of the remodeling transient. The rate of bone accumulation in this study was inversely proportional to age; the best estimate of the age at which the rate reached zero was approx 29 to 30 yr. Therefore, the window of opportunity to achieve the full genetic program appears to remain at least partly open until about age 30.

2.3. Secondary Prevention: The Conservation of Acquired Bone Mass

Studies of calcium requirement in mature, but premenopausal, women generally have yielded results compatible with the newer recommendations shown in Table 1. In a meta-analysis of studies in this age group, Welten et al. *(29)* concluded that calcium intake was positively associated with bone mass. In a study of estrogen-replete women ingesting their habitual calcium intakes, Heaney et al. *(30)* found zero calcium balance at a mean intake slightly less than 1000 mg/d. By contrast, Nordin et al. *(2)* found a figure closer to 600 mg/d, *and* in a prospective study of bone mass in premenopausal women, Recker et al. *(31)* found no detectable bone loss over a 2-yr period on an estimated mean calcium intake of 651 mg. (Corresponding RDAs, meeting the requirement of 95% of all individuals, were between approx 800 and 1200 mg/d.)

In conclusion, although there may be other health reasons for maintaining an even higher calcium intake during the mature years, bone health appears to be supported adequately by a calcium intake in the range of 800 to 1200 mg/d; lower intakes may lead to premenopausal bone loss, failure to achieve peak mass, or both.

2.4. Menopause

Estrogen has a bewildering variety of actions. In bone, it seems to adjust the bending set-point of the mechanical feedback loop that regulates bone mass. (The bending set-point is the amount of bending a bone experiences during loading that is sufficient to trigger a bone remodeling response.) Accordingly, whenever women lose ovarian hormones, either naturally at menopause or earlier as a result of anorexia nervosa or athletic amenorrhea, the skeleton appears to sense that it has more bone than it needs and, therefore, allows resorption to carry away more bone than formation replaces. (The same change occurs when men lose gonadal hormones for any reason.) This amounts to raising the set-point of the mechanical feedback loop that functions to maintain bone bending under load within safe limits. Although it varies somewhat from site to site across the skeleton, the downward adjustment in bone mass caused by lack of gonadal hormone at the spine amounts to approx 12 to 15% of the bone a woman had prior to menopause *(32)*.

The importance of this phenomenon in a discussion of nutrient effects is to distinguish menopausal bone loss from nutrient deficiency loss and to stress that menopausal loss, which mainly results from absence of gonadal hormones and not from nutrient deficiency, cannot be substantially influenced by diet. Almost all of the published studies regarding calcium supplementation within 5 yr following menopause failed to prevent bone loss. Even Elders et al. *(33)*, who employed a calcium intake in excess of 3100 mg/d, succeeded only in slowing menopausal loss and not in preventing it. Only a few reports, such as the study of Aloia *(19)*, contain clear evidence for a benefit of a high calcium intake at this life stage;

even in that study, estrogen produced a greater effect. Nevertheless, many of the published reports show evidence of small calcium effects even at this life stage, and it may be that in any group of early menopausal women, there are some whose calcium intake is so inadequate that they are losing bone for two reasons: estrogen lack plus calcium insufficiency.

Despite the importance of menopausal bone loss, it is only a one-time, downward adjustment, and if nutrition is adequate, the loss continues for only a few years, after which the skeleton is in a new steady state (although at a 12–15% lower bone mass). In this context, the importance of achieving a high peak skeletal mass during growth becomes apparent. One standard deviation (SD) for lumbar spine bone mineral content in normal women is about 12 to 15% of the young adult mean; for total body bone mineral, 1 SD is about 10 to 12%,. Hence, a woman at least 1 SD above the mean can sustain the 12 to 15% menopausal loss and still end up with about as much bone as the average woman has before menopause. In contrast, a woman at or below 1 SD below the young adult mean before menopause drops to 2 SDs below the mean as she crosses menopause and, therefore, by the World Health Organization criteria *(34)*, is already osteopenic and verging on frankly osteoporotic.

As noted, the menopausal bone mass adjustment theoretically stops with a loss of about 15%; however, this is true only when calcium intake is adequate. Therefore, it is important to note that estrogen has nonskeletal effects as well (i.e., it improves intestinal calcium absorption and renal calcium conservation) *(32,35,36)*. As a result, an estrogen-deficient woman has a higher calcium requirement, and unless she raises her calcium intake after menopause, she continues to lose bone after the estrogen-dependent quantum is lost, even if the same diet would have been adequate to maintain her skeleton before menopause. In other words, early in the menopausal period, her bone loss is mainly (or entirely) a result of estrogen withdrawal, whereas later it is a result of of inadequate calcium intake. Figure 4 assembles, schematically, the set of factors contributing to bone loss in the postmenopausal period. The figure shows both the self-limiting character of the loss resulting from estrogen deficiency and the usually slower, but progressive, loss resulting from nutritional deficiency (if present). Unlike the estrogen-related loss, which mostly plays itself out in 3 to 6 yr, an ongoing calcium deficiency loss continues to deplete the skeleton indefinitely for the remainder of a woman's life—that is, unless calcium intake is raised to a level sufficient to stop it. Furthermore, because both absorption efficiency *(35)* and calcium intake *(37)* decline with age, the degree of calcium shortfall actually tends to worsen with age.

Therefore, it is important for a woman to increase her calcium intake after menopause. Both the 1984 NIH Consensus Conference on Osteoporosis and the 1994 Consensus Conference on Optimal Calcium Intake *(8)* recommended calcium intakes of 1500 mg/d for estrogen-deprived postmenopausal women. The "Adequate Intakes" of the Food and Nutrition Board *(9)* for everyone over age 50 yr, when translated into RDA format (Table 1), are nearly identical (1450 mg/d). It may be that the optimal intake is somewhat higher (*see* Subheading 2.5.), but median intakes in the United States for women of this age are in the range of 500–600 mg/d *(37,38)*; if the bulk of them could be raised even to 1500 mg/d, the impact on skeletal health would be considerable.

2.5. Senescence

Age-related bone loss occurs in both sexes, regardless of gonadal hormone levels, generally starting at about age 50 yr. However, it is obscured in the years immediately

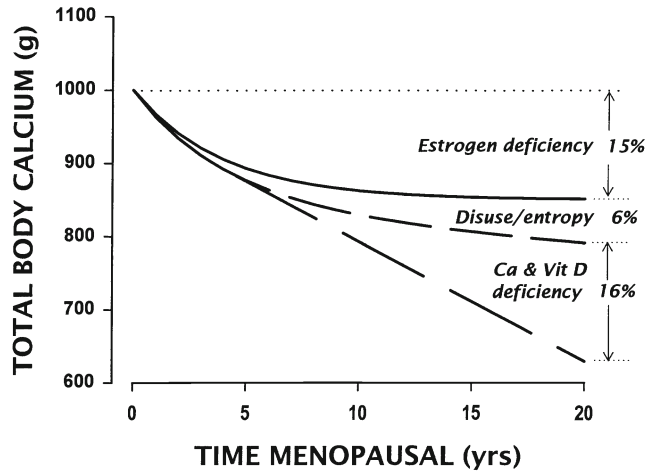

Fig. 4. Partition of age-related bone loss in a typical postmenopausal woman with an inadequate calcium intake. Based on a model described in detail elsewhere *(32)*. (Copyright Robert P. Heaney, 1990. Reproduced with permission.)

following menopause in women by the substantially larger effect of estrogen withdrawal (*see* Fig. 4). Nevertheless, it probably occurs (even in women treated with estrogen) at about the same rate as in men. This rate is generally reported to be approx 0.5 to 1.0% per year during the sixth and seventh decades of life and then accelerates with advancing age. For example, in the study by Chapuy et al. *(13)*, loss from the hip in the control subjects (average age: 84 yr) was 3% per year,. Age-related loss involves both cortical and trabecular bone and can occur through several mechanisms (disuse, remodeling errors, and nutritional deficiency), which are summarized in Fig. 4.

Although nutrient deficiency clearly is only a part of the total problem, it is common. The relationship between nutritional status and the 3% loss in the control subjects in the study by Chapuy et al. . is indicated by the fact that this loss was completely obliterated in the calcium- and vitamin D-supplemented women. Intestinal calcium absorption efficiency declines with age *(35)* at the same time as nutrient intake generally declines *(37)*; the result is that the diet of aging individuals becomes more and more inadequate. McKane et al. *(39)* showed that the high PTH levels and abnormal PTH secretory dynamics typically found in elderly women result from calcium deficiency and that PTH function can be entirely normalized by calcium intakes of 2400 mg/d. In a somewhat younger group of elderly subjects, Peacock et al. *(40)* showed that elevating calcium intake from approx 550 to 1300 mg/d effectively obliterated bone loss at the hip and total body over a 4-yr treatment period.

Also, it is in the elderly age group that the most dramatic and persuasive evidence for fracture prevention by high calcium intakes has been produced *(13,17,41,42)*. This is partly because most fragility fractures rise in frequency with age, and thus the opportunity to see a fracture benefit (if one exists) is greater in elderly individuals. Chapuy et al. *(13)* showed a reduction in hip fracture risk of 43% and a 32% reduction in other extremity fractures by 18 mo after starting supplementation with calcium plus vitamin D. Dawson-Hughes et al. *(41)* produced a greater than 50% reduction in all fractures by 36

mo of supplementation with both calcium and vitamin D. In another study in elderly women, Chevalley et al. *(17)* showed that even when vitamin D was given to both groups, extra calcium reduced femoral bone loss *and* vertebral fracture incidence. In a 4-yr, RCT in elderly women (mean age: 73 yr), Recker et al. *(42)* demonstrated that a calcium supplement reduced both age-related bone loss and incident vertebral fractures. Their subjects all had received a multivitamin supplement as had the subjects of Chevalley et al. *(17)*; hence, the effect in the calcium-supplemented groups of both studies can be attributed to the extra calcium alone.

These findings do not suggest that vitamin D is unimportant in this age group (*see* Subheading 3.). It is likely that intakes of both calcium and vitamin D are inadequate in the elderly, and the unrecognized prevalence of combined deficiency has made it difficult to study the actual requirements of either nutrient in this age group.

The calcium intakes achieved in the studies by Chapuy et al. *(13)*, Chevalley et al. *(17)*, Dawson-Hughes et al. *(41)*, and Recker et al. *(42)* were about 1700, 1400, 1300, and 1600 mg/d, respectively. These values are in the range of the intake found by Heaney et al. *(30)* to be the mean requirement for healthy estrogen-deprived older women (1500–1700 mg/d). Therefore, all these studies are congruent with the newer recommendations of 1400 to 1500 mg/d (Table 1).

It has been generally considered that the antifracture benefit of calcium observed in these controlled trials resulted from protection against calcium deficiency-induced bone loss. However, the fracture benefit is substantially larger than the bone mass difference predicts, and the fracture reduction is apparent within a few months of starting calcium (and vitamin D) supplementation *(13,41,43)*—well before much effect on bone mass could occur. A better explanation seems to be reduction in the excessively high remodeling activity that is common in postmenopausal women and generally in the elderly. Remodeling activity is emerging as an important risk factor for fragility, and any agency that reduces remodeling seems to reduce fracture risk independently of an effect on bone mass *(43)*.

An important feature of these controlled trials in already elderly individuals was that bone mass was low in both treated and control groups at the start of the study, and although a significant difference in fracture rate was produced by calcium supplementation, even the supplemented groups displayed what would have to be considered as an unacceptably high fracture rate. These studies do not establish how much lower the fracture rate might have been if a high calcium intake had been provided for the preceding 20 to 30 yr of these women's lives. The studies of Matkovic et al. *(44)* and Holbrook et al. *(45),* although not randomized trials, strongly suggest that the effect may be larger than has been found with treatment started in the seventh to ninth decades of life. Both of these observational studies reported a hip fracture rate that was roughly 60% lower in elderly persons whose habitual calcium intakes had been high. Although findings from observational studies such as these had not been considered persuasive in the absence of proof from controlled trials, many controlled trials have shown a skeletal benefit of added calcium and have removed that uncertainty.

The aggregate of available studies underscores the importance of achieving at least the target figure of 1500 mg calcium for the elderly. It also must be stressed again that osteoporosis is a multifactorial condition and that removing one of the pathogenic factors (i.e., ensuring an adequate calcium intake) cannot be expected to eradicate all osteoporotic fractures.

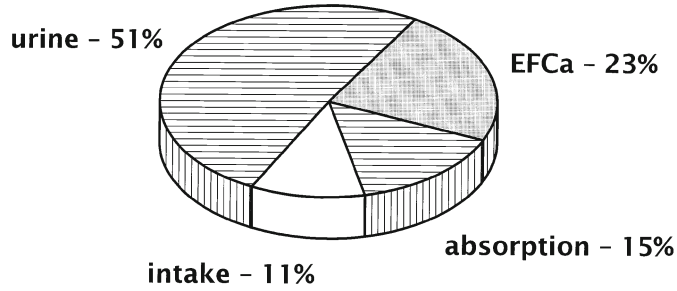

Fig. 5. Partition of variance in calcium balance in normal women among the input-output processes involved in calculation of balance. (Copyright Robert P. Heaney, 1994. Reproduced with permission.)

2.6. Nutrient–Nutrient Interactions: Factors That Influence the Requirement

There are several nutritional factors that influence or have been proposed to influence the calcium requirement. These are probably less important than once believed, because at calcium intakes in the ranges currently recommended, interactions tend to have negligible impact on the calcium economy. Nevertheless, the issue of interactions remains, and, therefore, we briefly address the issue in this section.

The principal interacting nutrients are sodium, protein, caffeine, and fiber. Fiber and caffeine influence calcium absorption *(46–49)* and typically exert relatively minor effects, whereas sodium and protein influence urinary excretion of calcium *(49,50)* and can be of much greater significance for the calcium economy when calcium intakes are low. The net effects of phosphorus and fat in humans are minor to nonexistent.

The basis for the differing importance of these nutritional factors on the calcium economy is illustrated in Fig. 5, which partitions the variance in calcium balance observed in 560 balances in healthy middle-aged women consuming typical intakes and studied in the author's laboratory. As Fig. 5 illustrates, only 11% of the variance in balance among these women is explained by differences in their actual calcium intakes. In contrast, absorption efficiency explains about 15%, and urinary losses explain more than 50%.

2.6.1. Influences on Intestinal Absorption of Calcium

2.6.1.1. Fiber. The effect of fiber is variable and generally small. Many kinds of fiber have no influence at all on absorption, such as the fiber in green, leafy vegetables *(10)*. In contrast, the fiber in wheat bran reduces absorption of coingested calcium, although with the exception of extremes of fiber intake *(51)*, the overall effect generally is relatively small. Often associated with fiber are related plant food constituents such as phytate and oxalate. Both can reduce the availability of any calcium contained in the same food; however, unlike bran, phytate and oxalate generally do not affect coingested calcium from other foods. For example, for equal ingested loads, the calcium of beans is only about half as available as the calcium of milk *(52)*, whereas the calcium of spinach and rhubarb is nearly totally unavailable *(53)*. For spinach and rhubarb, the inhibition is mostly a result of oxalate. For common beans, phytate is responsible for about half the interference, and oxalate is responsible for the other half. Even so, the effects of phytate and oxalate are highly variable among foods. There is a sufficient

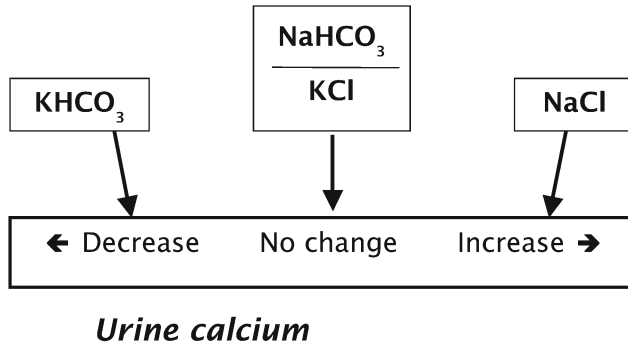

Fig. 6. Effects of various sodium and potassium salts on urine calcium. (Copyright Robert P. Heaney, 2003. Used with permission.)

quantity of both antiabsorbers in beans to complex all the calcium also present, and yet absorptive interference is only half that expected.

2.6.1.2. Caffeine. Although caffeine is often considered to have a deleterious effect on the calcium economy, it actually has the smallest effect of the known interacting nutrients *(47)*. A single cup of brewed coffee causes deterioration in calcium balance of approx 3 mg *(48,49,54)*, mainly by reducing absorption of calcium *(48)*. The effect is probably on active transport, although this is not known with certainty. This small effect is more than adequately offset by 1–2 tablespoons of milk *(48,54)*.

2.6.2. Influences on Renal Conservation of Calcium

2.6.2.1. Protein and Sodium. As noted earlier, the effects of protein and of sodium can be substantial *(2,3,49)*. Both nutrients can increase urinary calcium loss across the full range of their own intakes, from very low to very high; thus, the concern is not solely for the harmful effects of an *excess* of these nutrients. Sodium and calcium share the same transport system in the proximal tubule, and every 2300 mg of sodium excreted by the kidney pulls 20 to 60 mg of calcium out with it. Additionally, every gram of typical protein (whether from animal or vegetable sources) metabolized in adults causes an increment in urine calcium loss of about 1 mg. This latter effect probably results from excretion of the sulfate load produced in the metabolism of sulfur-containing amino acids (and is thus a kind of endogenous analog of the acid rain problem).

Although much of the literature in this field stresses the role of sodium, it is important to recognize that most dietary sodium is in the form of table salt (NaCl) and that the often ignored anion plays an important role in these interactions. This issue is too complex for exhaustive treatment in this chapter, and it is sufficient to note only that sodium bicarbonate does not have the same hypercalciuric effect as sodium chloride *(55)* and that potassium bicarbonate completely obliterates the calciuria of a high NaCl intake *(56)*. Figure 6 summarizes the interplay of sodium, potassium, and their respective anions on urine calcium.

At low salt and protein intakes, the minimum calcium requirement for an adult premenopausal female may be as little as 450 mg/d *(50)*, whereas if her intake of both nutrients is high, she may require as much as 2000 mg/d to maintain calcium balance. A forceful illustration of the importance of sodium intake is provided by the report of

Matkovic et al. *(57),* which *shows* that urine calcium remains high in adolescent girls on calcium intakes too low to permit bone gain. The principal determinant of urinary calcium in such young women is sodium intake *(58)*, not calcium intake.

Differences in protein and sodium intake among national groups are perhaps part of the reason that studies in different countries have sometimes shown strikingly different calcium requirements. At the same time, there is usually a positive correlation between calcium intake and bone mass within each national range of intakes *(59)*. Therefore, although sodium (and protein) intake differences between cultures obscure the calcium effect, they do not obliterate it.

For diets high in calcium (as was the case for our hunter–gatherer ancestors), high protein and, possibly, high sodium intakes could have been handled perfectly well by the body. At the low intakes that prevail today, an individual's absorptive performance is close to maximal. Augmented loss from increased sodium or protein intake cannot be offset by increasing extraction from the diet, because there is less in the food to extract and because extraction efficiency is already at the upper end of its possible range. In contrast, at intake levels typical of those that prevailed during hominid evolution, intestinal absorption is predominantly passive, and the full range of absorptive adaptation is available to offset increased excretory or cutaneous losses. In brief, these nutrients create problems for the calcium economy of contemporary adult humans mainly because we typically have calcium intakes that are low and sodium intakes that are high relative to those of preagricultural humans.

2.6.2.2. Acid Ash Residue. The acid/alkaline ash characteristic of the diet also may be important, although to date, the quantitative relationship of this diet feature to the calcium requirement has been less fully explored. Nevertheless, it has clearly been shown that substitution of metabolizable anions (e.g., bicarbonate or acetate) for fixed anions (e.g., chloride) in various test diets substantially lowers obligatory urinary calcium loss *(60,61)*. This suggests that primarily vegetarian diets create a lower calcium requirement and provides a further explanation for the seemingly lower requirement in many nonindustrialized populations. However, it is not yet clear whether vegetarians have higher bone mass values than omnivores within a population, and some data suggest that they may actually have less dense skeletons, possibly because of the commonly very low calcium levels of such diets *(62,63)*.

2.6.2.3. Phosphorus. Phosphorus is commonly believed to reduce calcium absorption, but the evidence for that effect is scant to nonexistent, and there is much contrary evidence. In analysis of 567 metabolic balances performed in healthy middle-aged women studied on their usual diets, variation in phosphorus intake over a nearly sixfold range had no detectable effect on calcium absorption efficiency *(64)*. In a controlled metabolic study, Spencer *(65)* found no effect of even large increments in phosphate intake on overall calcium balance at low, normal, and high intakes of calcium. In adults, calcium to phosporus ratios (Ca:P) ranging from 0.2 to more than 2.0 are without effect on calcium balance, at least so long as adjustments are made for calcium intake *(49,64)*.

Phosphorus does depress urinary calcium loss and elevate digestive juice secretion of calcium by approximately equal amounts, with little or no net effect on balance *(66)*. Although it is true that stoichiometric excesses of phosphate tend to form complexes with calcium in the chyme, various calcium phosphate salts have been shown to exhibit absorbability similar to other calcium salts, and phosphate is, of course, a principal anion of the major food source of calcium (dairy products). In any case, phosphate is more

readily absorbed than calcium (by two to five times), and at intakes of both nutrients in the range of their respective RDAs, absorption leaves a stoichiometric excess of calcium in the ileum, not vice versa. This explains the apparent paradox that high calcium intakes can block phosphate absorption (as in management of end-stage renal disease), whereas achievably high phosphorus intakes have little or no effect on calcium absorption.

2.6.2.4. Aluminum. Although aluminum (Al) is not in any proper sense a nutrient, in the form of Al-containing antacids, it exerts significant effects on obligatory calcium loss in the urine *(67)*. By binding phosphate in the gut, these substances reduce phosphate absorption, lower integrated 24-h serum phosphate levels, and, thereby, elevate urinary calcium loss. (This is the opposite of the more familiar hypocalciuric effect of oral phosphate supplements.) Therapeutic doses of Al-containing antacids can elevate urine calcium by 50 mg/d or more.

2.7. Calcium Sources

The best calcium sources are, of course, foods. In a modern, Western diet, food items that provide more than 100 mg of calcium per serving are limited to dairy products (with the exception of cottage cheese), greens of the mustard family (collards, kale, mustard), calcium-set tofu, sardines, and a few nuts (especially hazelnuts and almonds). Smaller amounts of calcium are ubiquitous in many leafy vegetables; however, with the exception of shellfish, calcium levels are low in most meats, poultry, or fish. As noted earlier, the calcium of beans is only about half as available as the calcium of milk, and the calcium of high-oxalate vegetables (such as spinach and rhubarb) is almost completely unavailable. Figure 7 displays the available calcium in a variety of foods. "Available" represents the product of the fractional absorbability of the calcium in a food and its total calcium content. Therefore, "available" refers to the actual, gross* amount of calcium a particular food delivers into the blood of the absorbing subject.

In general, most diets without dairy products have total calcium nutrient densities below 20 mg of calcium per 100 kCal, and available calcium densities are even lower. Because total energy intake for adult American women is approx 1800 kCal/d, it follows that most diets low in dairy products are low in calcium (probably 300–400 mg calcium/d or less) andfar short of levels currently considered optimal.

In part as a response to this dilemma, the surgeon general, in his 1988 report on Nutrition and Health *(68)*, recommended judicious, low-level calcium fortification of many items in the food chain. An increasing number of fortified foods are becoming available each year, ranging from fruit juice to bread, to breakfast cereals, to potato chips, to rice. Where bioavailability of added calcium has been assured, these foods should be useful adjuncts in the attempt to improve calcium intake at the population level. However, satisfactory bioavailability does not automatically follow from simply adding calcium to a product. Soy beverage fortified to the calcium load of cow milk was shown to deliver only 75% the amount of calcium as cow milk *(69)*, and recent entries into the soy and rice beverage markets as well as several of the calcium-fortified orange juices exhibited even poorer physical characteristics *(70)*.

The principal calcium supplement in the US market is calcium carbonate, available alone or as oyster shell or dolomite. When the tablet is competently formulated (so that it

*In the process of digestion, substantial quantities of calcium enter the gut in the form of digestive secretions and sloughed mucosa. For this reason, net absorption is always less than gross.

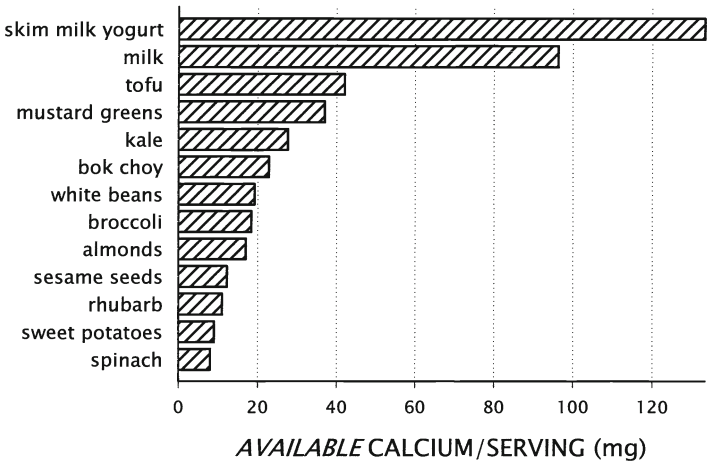

Fig. 7. Available calcium per serving for a variety of foods. "Available calcium" is the product the calcium content of a food and its fractional absorbability. (Copyright Robert P. Heaney, 1998. Reproduced with permission.)

disintegrates in gastric juice) or when the supplement is chewed, the carbonate is well-absorbed and generally very well-tolerated. There is no requirement for gastric acid *per se*, so long as the carbonate salt is taken with meals. Calcium citrate and calcium citrate malate (CCM) also are good sources, but they tend to be more expensive *(71)*. In some studies, CCM was reported to exhibit higher absorption efficiency than calcium carbonate *(41)*; the study by Peacock et al. *(40)*, using CCM as the calcium source, reported one of the largest calcium effects on age-related bone loss observed to date. In the rare case that the carbonate seems not well-tolerated, these other sources provide useful alternatives.

Divided doses enhance absorption from both supplements and foods, because absorption fraction is an inverse function of load size. All calcium sources (including food) interfere with iron absorption when the two nutrients are ingested at the same meal. However, single-meal studies miss the body's upregulation of iron absorption in the face of need, and chronic feeding studies have revealed no deterioration of iron status in subjects consuming high-calcium diets. Perhaps of greater relevance, Matkovic and colleagues *(72)* have shown convincingly that adolescent girls are able to increase total body iron stores normally in the presence of 1600-mg calcium intakes. This is a particularly reassuring finding, because females in this age group currently are among the most vulnerable to iron deficiency in the United States. However, if an adult is iron-deficient (e.g., as a result of severe blood loss) and is taking an iron supplement, it may be best if the meal at which the iron is taken does not contain a large amount of calcium (in food or supplement form).

3. VITAMIN D

Vitamin D facilitates active transport of calcium across the intestinal mucosa, partly by inducing the formation of a calcium-binding transport protein in intestinal mucosal cells. This function is particularly important for adaptation to low calcium intakes. Absorption also occurs passively, probably mainly by way of paracellular diffusion. This route is not dependent on vitamin D and has not been studied as well. The proportion of absorption by the two mechanisms varies with intake and is not well-characterized

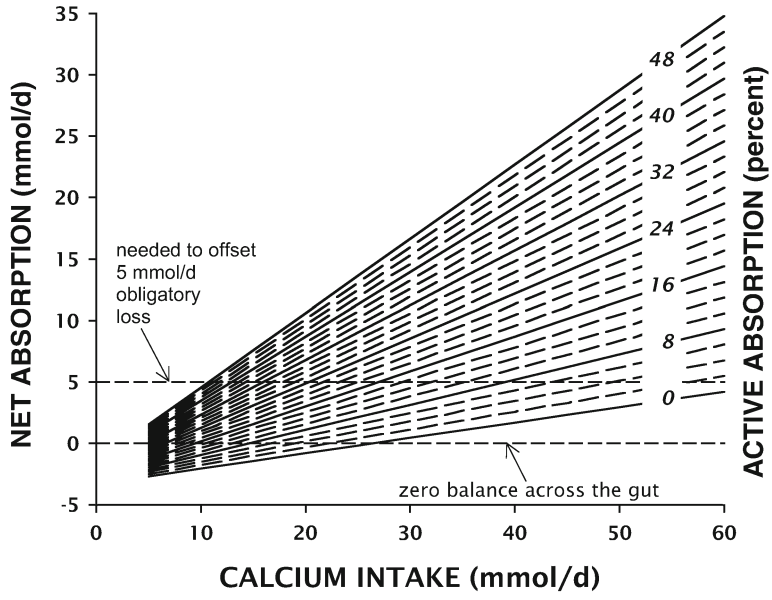

Fig. 8. Relationship of vitamin D-mediated, active calcium absorption, calcium intake, and net calcium gain across the gut. Each of the contours represents a different level of active absorption above a baseline passive absorption of 12.5%. (The values along each contour represent the sum total of passive and variable active absorption.) The horizontal dashed lines indicate 0 and 5 mmol/d (200 mg/d) net absorption, respectively. The former is the value at which the gut switches from a net excretory to a net absorptive mode, and the latter is the value needed to offset typical urinary and cutaneous losses in mature adults. (Copyright Robert P. Heaney, 1999. Reproduced with permission.)

in humans; at high calcium intakes (>2400 mg/d), absorption fraction approaches 10 to 15% of intake. Under these circumstances, it is likely that active transport contributes relatively little to the total absorbed load. Nevertheless, it generally is considered that vitamin D status regulates absorptive performance and thereby influences the calcium requirement. The quantitative importance of active transport of calcium at prevailing calcium intakes is illustrated in Fig. 8, which plots the actual net quantity of calcium absorbed as a function of both calcium intake and active absorption. As demonstrated in Fig. 8, some degree of active absorption is needed to offset excretory losses, even at calcium intakes as high as 50 mmol (2000 mg)/d.

A principal storage form of the vitamin is 25-hydroxyvitamin D [25(OH)D], and its plasma level is generally regarded as the best clinical indicator of vitamin D status. Although usually considered to be about three orders of magnitude less potent than calcitriol in promoting active transport in animal receptor assays, there is growing evidence that it may possess physiological functions in its own right *(73,74)*; in the only human dose–response studies performed to date, 25(OH)D was found to have a molar potency in the range of 1/100 to 1/125 that of $1,25(OH)_2D_3$ *(75,76)*—not the 1/2000 amount usually considered to reflect relative 25(OH)D activity.

Vitamin D status commonly deteriorates in the elderly, in whom plasma 25(OH)D levels are generally lower than in young adults *(77)*. This difference results partly from

decreased solar exposure, partly from decreased efficiency of skin vitamin D synthesis, and partly from decreased intake of milk, the principal dietary source of the vitamin. Moreover, the elderly exhibit other abnormalities of the vitamin D endocrine system that may further impair their ability to adapt to reduced calcium intake. These include decreased responsiveness of the renal 1-α-hydroxylase to PTH *(78)* and, possibly, decreased mucosal responsiveness to calcitriol *(79)*.

For these reasons, there is a growing consensus that the requirement for ingested vitamin D rises with age *(9)* and a body of data that strongly suggests that relative vitamin D deficiency plays a role in several components of the osteoporosis syndrome. Although a trial by Lips et al. *(80)* regarding vitamin D supplementation noted no fracture reduction, others demonstrated clear benefits from supplementing vitamin D in the elderly. One example is the RCT by Heikinheimo et al. *(81)*, which demonstrated a significant reduction in all fractures in an elderly Finnish population given a single annual injection of 150,000 to 300,000 IU of vitamin D each fall (equivalent to approx 400–800 IU/d). The impressive fracture reductions noted in the trials of Chapuy et al. *(13)* and Dawson-Hughes et al. *(41)* may also have partly resulted from the vitamin D supplementation that was a component of both trials. Most recently, in a large prospective study of British elderly subjects, vitamin D at an average dose of approx 820 IU/d reduced typical osteoporotic fracture risk by 33% *(82)*.

The foregoing studies (as well as others *[83,84]*) strongly suggest that vitamin D insufficiency is prevalent in the middle-aged and elderly of northern Europe and North America. In one study of north Italian centenarians, severe vitamin D deficiency was virtually universal *(85)*. However, with the exception of this latter study, frank osteomalacia was not reported to be a significant feature of the clinical status of study participants. This old criterion for true vitamin D deficiency is much too insensitive to be clinically useful today, since it misses most of the cases of clinically important vitamin D deficiency.

The issue of how the vitamin D requirement should be defined is another matter. Holick *(86)* showed that it takes an intake of at least 600 IU/d, from all sources, to sustain serum 25(OH)D levels in healthy young adults, and the doses of vitamin D used in the studies summarized earlier also suggest that an intake in the range of 500 to 800 IU/d is required for full expression of the known effects of vitamin D in adults. This is substantially greater than the RDA from 1989 of only 200 IU for adults *(21)*. The new DRIs for vitamin D *(9)* reflect this realization, if only to a limited extent. They included an increase from 200 to 400 IU for adults ages 50 to 70 yr and to 600 IU for those over age 70. Vieth *(87)* presented evidence that the requirement may be higher still. In controlled dosing studies, my colleagues and I *(88)* showed that the body normally uses approx 4000 IU (100 µg)/d, most of which must come from cutaneous sources. This same study showed that serum 25(OH)D rose by about 0.7 nmol/L for every 1 µg daily input of cholecalciferol. Other studies *(80,87)* provided estimates of rates of increase ranging up to 1.2 nmol/L per microgram of daily input. Taking a value between these extremes (1 nmol/L per microgram), it follows that an individual with a serum 25(OH)D of 50 nmol/L will need an additional 30 µg (1200 IU) of cholecalciferol daily to achieve a stable level of 80 nmol/L.

Ascertaining the vitamin D requirement is complicated by the mixed input of cutaneous and dietary vitamin D. Because skin production under ambient conditions is largely unknown, it is difficult to estimate how much must be provided orally in those

who are housebound or excluded from skin exposure to solar radiation; however, it could be as much as 4000 IU (100 μg)/d.

4. PROTEIN

Two seemingly contradictory facts seem well-established regarding calcium and protein: (a) protein increases urinary calcium loss *(49,89,90)*; and (b) protein aids recovery from hip fracture *(91,92)* and slows age-related bone loss *(92,93)*. For the most part, the studies establishing these diverse and, to some extent, contradictory effects were performed by varying only the nutrient concerned. For example, the calciuric effects of protein were demonstrated most clearly in studies in which purified protein or protein hydrolysates were used, with each gram of protein resulting in an approximate rise of 1 mg in urinary calcium excretion. However, Spencer observed that when the protein was fed as ground beef, urinary calcium did not rise, with the difference in response presumably resulting from the fact that the meat contained substantial quantities of phosphorus *(94)*.

Kerstetter et al. *(95)* reported that high protein intakes enhanced calcium absorption, an effect that would counter a calciuric effect (if there were such). Not everyone has been able to reproduce this finding in chronic feeding studies *(64)*, and it may, to the extent that the phenomenon is operative, apply only acutely. In a very recent controlled feeding study, Roughead et al. *(96)* reported no effect of high and low protein intakes from meat (117 vs 68 g/d) on either calcium balance or urinary calcium excretion, a finding consistent with that of Spencer et al. *(94)* and Heaney and Recker *(64)* but at odds with that of Kerstetter *(95)*.

The very reproducible calciuric effect of pure protein or amino acids led, several years ago, to the tentative conclusion that high protein intakes might be deleterious for the skeleton. However, not only do the studies involving food sources of protein (such as those just cited) not support that conclusion, but epidemiological studies (such as those from the Framingham osteoporosis cohort *[93]*) indicate that age-related bone loss in postmenopausal women is inversely related to protein intake rather than directly related, as might have been predicted from the calciuric effect.

In this connection, it is interesting to examine the interaction of protein and calcium intakes in the study of Dawson-Hughes and colleagues *(97)*. In their randomized controlled trial of calcium supplementation, the bone gain associated with calcium supplementation was confined to individuals in the highest tertile of protein intake, whereas in the placebo group, there was a nonsignificant trend toward worsening bone status as protein intake rose. This latter effect is what would be predicted if there were some degree of protein-induced calciuria without an offsetting increase in absorbed calcium. Protein intake in this study spanned only a relatively narrow range and was not randomly assigned to the subjects; therefore, these results cannot be considered final. Nevertheless, they do exhibit two interesting features: (a) high protein intake clearly did not block the positive effect of calcium; and (b) most of the protein was from animal sources (as was also true for the Framingham osteoporosis cohort). This latter point provides no support for the hypothesis that animal foods (in contrast with vegetable protein sources) artificially elevate the calcium requirement by increasing urinary calcium loss.

The importance of achieving an adequate protein intake is at least twofold. First, protein is a bulk constituent of bone. Because of extensive posttranslational modification of the collagen molecule (e.g., crosslinking, hydroxylation, etc.), many of the

amino acids released in bone resorption cannot be recycled. Hence, bone turnover requires a continuing supply of fresh protein. Second, protein elevates serum insulin-like growth factor (IGF)-1 *(98,99)*, which is trophic for bone *(99)*. For both reasons, a diet inadequate in protein would be expected to reduce the bony response to calcium. Moreover, several studies of increased protein intake showed rises in serum IGF-1 *(98,99)*. Thus, it may be tentatively concluded that protein intakes in the individuals concerned were suboptimal. To the extent that this is true and that both of the bulk constituents of bone (calcium and protein) are ingested at suboptimal intakes, it follows that the true effect of neither nutrient can be discerned in studies that do not ensure full repletion of the other.

The IGF-1 response is of particular interest. IGF-1 rises with protein intake; but above certain protein intakes, no further increase in IGF-1 can be produced. This effect is analogous to calcium retention, which rises at suboptimal calcium intakes but plateaus at or above the individual's calcium intake requirement. For both nutrients, a rise in retention (for calcium) or a rise in IGF-1 (for protein) can be taken as evidence that the presupplement intake of the corresponding nutrient was suboptimal. The fact that IGF-1 has not reached its plateau at intakes in the range of current RDAs has led some to conclude that the current RDA for protein is set too low, particularly for the elderly. In any case, it is important to understand this relationship of nutrient intakes used in various studies to the respective plateaus. Different studies are expected to show more or less of a beneficial effect of increasing one or both nutrient intakes, depending on whether the presupplement intake were below or above the respective threshold level.

5. VITAMIN K

The chemistry and physiology of vitamin K have been reviewed extensively elsewhere *(100,101)*. In brief, vitamin K is necessary for the γ-carboxylation of glutamic acid residues in a large number of proteins. Most familiar are those related to coagulation, in which seven vitamin K-dependent proteins are involved in one way or another. The γ-carboxyglutamic acid residues in the peptide chain bind calcium, either free or on the surface layers of crystals, and have been thought to function in varying ways, including catalysis of the coagulation cascade, inhibition of mineralization (as in urine), and generation of osteoclast chemotactic signals.

Three vitamin K-dependent proteins are found in bone matrix: osteocalcin (bone gla protein[BGP]), matrix gla-protein, and protein S. Only BGP is unique to bone. There also is a kidney gla protein (nephrocalcin), which may be involved in renal reabsorption of calcium. BGP binds avidly to hydroxyapatite and is chemotactic for bone-resorbing cells. Roughly 30% of the synthesized BGP is not incorporated into matrix but instead is released into the circulation, where, similarly to alkaline phosphatase, it can be measured and used as an indicator of bone turnover. In vitamin K deficiency, as occurs with coumarin anticoagulants, serum BGP levels decline, and the degree of carboxylation of the circulating BGP falls dramatically. Although it would therefore seem that vitamin K deficiency would have detectable skeletal effects, they have been very hard to demonstrate. Rats reared and sustained to adult life under near total suppression of BGP γ-carboxylation show only minor skeletal defects, mostly related to abnormalities in the growth apparatus *(100)*. In aging humans, the problem of detecting skeletal abnormalities is compounded by the fact that the bulk of the skeleton was formed prior

to the onset of any deficiency, and thus bone tends to be an insensitive indicator of current nutritional stresses.

Various vitamin K-related abnormalities have been described in association with osteoporosis, but their significance to skeletal status remains unclear. Women with low dietary vitamin K intakes have significantly lower values for bone mineral density at hip and spine *(102)* and greater risk for hip fracture *(103)* than do those with higher intakes . Circulating vitamin K and menaquinone levels are low in hip fracture patients *(104)*. BGP is under-carboxylated in osteoporotics, and this defect responds to relatively small doses of vitamin K. However, maximal suppression of under-carboxylation has been reported to require in excess of 1000 μg of vitamin K per day *(105)*. Finally, urine calcium has been reported to be high in osteoporotics and to fall on administering vitamin K *(106)*.

Whether or not vitamin K is important for bone health, serum vitamin K levels are indicators of general nutritional status, and it may be that the observation of low vitamin K levels in osteoporotics, especially in those with hip fracture, is mainly a reflection of the often poor general nutrition of these individuals.

6. MAGNESIUM

The adult female RDA for magnesium was 280 mg/d in the 1989 RDAs *(21)* and revised upward to 320 mg/d in the 1997 DRIs *(9)*. Only about 25% of adult females in the United States achieve this level of intake on any given day. Average intakes tend to be in the range of 70 to 80% of the RDA. Although severe magnesium deficiency is a well-described syndrome *(107)*, interfering both with PTH secretion and PTH action on bone, it is uncertain whether mild departures from the RDA have any adverse effect or even whether the RDA needs to be as high as it is now set. There is also a widespread popular belief that magnesium is necessary for optimal calcium absorption. However, the many studies described earlier that established the benefit of supplemental calcium achieved their effect without adding magnesium to the diets of their subjects. Furthermore, Spencer *(108)*, in a series of careful metabolic studies, showed that a tripling of magnesium intake had no effect on absorption efficiency for calcium. Thus, there is no known justification for supplemental magnesium in prevention or treatment of osteoporosis. Moreover, magnesium salts, when used as a component of a combined supplement tablet (e.g., as in dolomite), displace calcium and make it more difficult (i.e., more pills are required) to get sufficient calcium by this route.

However, an unknown but probably small proportion of patients with osteoporosis have silent celiac disease as a contributory factor in their disease. These individuals commonly have some degree of magnesium deficiency. Because the underlying problem in such cases is asymptomatic, it usually is unrecognized and, therefore, untreated. For that reason, there may well be a small group of osteoporotic patients who would benefit from supplemental magnesium (as well as from calcium and vitamin D).

7. TRACE MINERALS

Several trace minerals (notably zinc, manganese, and copper) are essential metallic cofactors for enzymes involved in synthesis of various bone matrix constituents. Ascorbic acid (along with zinc) is needed for collagen crosslinkage. In growing animals, diets

deficient in these nutrients produce clear-cut skeletal abnormalities *(109)*. Additionally, zinc deficiency is well-known to produce growth retardation and other abnormalities in humans. However, it is not known whether significant deficiencies of these elements develop in previously healthy adults or, at least, if they do, whether such deficiencies contribute detectably to the osteoporosis problem. Copper deficiency is reported to be associated with osteoporotic lesions in sheep, cattle, and rats *(110)*. Copper has not been well-studied in connection with human osteoporosis; however, in one study in which serum copper was measured, levels were negatively correlated with lumbar spine bone mineral density, even after adjusting for body weight and dietary calcium intake *(111)*.

Copper-deficient animals develop reduced collagen crosslinks, a factor known to weaken bone strength. Oxlund *(112)* reported reduced extractable crosslinks in the bone of osteoporotic patients, but it is not known whether copper deficiency was the cause.

In one four-way, randomized trial, copper (as a part of a trace mineral cocktail including also zinc and manganese) slowed bone mineral loss in postmenopausal women when given either with or without supplemental calcium *(113)*. There appeared to be a small additional benefit from the extra trace minerals; however, the only statistically significant effect in this study was associated with the calcium supplement. This could mean that trace mineral deficiency plays no role in osteoporosis, but it could also mean that not all of the women treated suffered from such deficiency. In fact, because both osteoporotic and age-related bone loss are multifactorial and there is no known way to select subjects for inclusion on the basis of presumed trace mineral need, one might presume that only some of the subjects in such a study might be deficient. Thus, the suggestive findings of this study must be considered grounds for further exploration of this issue.

8. NUTRITION AND HIP FRACTURE

Nutrition enters into the hip fracture problem in two ways: (a) in predisposing to fracture; and (b) in recovery from the assault of the injury and its surgical repair. Fractures, particularly hip fractures, in the elderly are concentrated in institutionalized persons with multiple disabilities. Generally, the osteoporotic elderly are known to have depleted lean body mass and fat mass and, when studied, show low circulating values for several key nutritional indicator variables, from serum albumin to ferritin and vitamins A, D, and K *(91)*. Survival 2 yr after injury is four times higher in patients with serum albumin values over 3.5 g/dL than in patients with values below 3.0 g *(91)*. Additionally, patients with hip fracture often have low calcium intakes, and in the majority of studies evaluating the matter, dietary calcium earlier in life is inversely associated with hip fracture risk. In brief, hip fracture is a problem concentrated in multiply compromised individuals, and the prospect of successfully intervening to reduce risk has proved daunting even to contemplate.

However, one aspect of the problem is partly amenable to change. The relative malnutrition of patients suffering hip fracture and coming to hospital for repair contributes significantly to the often unsatisfactory outcomes for this common fracture (i.e., 15–20% excess mortality; 50% institutionalization of the survivors). In an RCT of a protein-based nutrient supplement given to patients newly hospitalized for hip fracture, Delmi et al. *(91)* found that only 26% of unsupplemented individuals had outcomes classified as "good" at 6 mo after injury, whereas nearly 60% of supplemented individuals had "good" outcomes. The investigators noted that the hospital diets offered to the

unsupplemented individuals were nutritionally adequate but were frequently unconsumed, while the investigators ensured the ingestion of the supplement. This is not an isolated observation; others *(114)* earlier found qualitatively similar benefit from nutritional supplementation in such patients. Additionally, protein supplements after fracture were shown to retard bone loss in the contralateral hip *(92)*. The consistency of these findings constitutes a challenge to the health professions to apply these basic nutritional principles in the management of their patients.

9. NUTRITION AND GLUCOCORTICOID-INDUCED OSTEOPOROSIS

Glucocorticoid-induced osteoporosis (GIO) can be one of the most disabling and rapidly progressing forms of the disorder. Severe compression fractures can occur in as little as 6 mo after starting corticosteroid therapy. The reasons are not entirely clear. Corticosteroids depress virtually all anabolic activities in the body, including osteoblastic new bone formation. Excessive bone resorption often occurs as well, which, when taken together with depressed bone formation, leads to negative bone balance. However, the rate of progression of GIO is too rapid to be explained on bone mass grounds alone. It is likely that accelerated remodeling, particularly in vertebral cancellous bone, directly compromises trabecular strength to an extent out of proportion to the loss in mass. Whatever the ultimate mechanism, current standards of practice call for cotherapy with a remodeling suppressor whenever glucocorticoids are prescribed for a chronic condition.

However, pharmacological prophylaxis of GIO does not negate the need for nutritional measures as well. Vitamin D status and calcium absorption commonly are low in patients taking corticosteroids, and both defects aggravate the tendency to increased remodeling and bone resorption. Sufficient vitamin D_3 should be given to raise the serum 25(OH)D level to 80 nmol/L (32 ng/mL), and calcium intake should be at least 1500 mg/d and perhaps as much as 2500 mg/d. These measures alone are not sufficient, but pharmacological prophylaxis alone is insufficient as well. Both are necessary.

10. RECOMMENDATION

Calcium intake should be high throughout life: 1500 mg/d during growth and at least that much in the elderly. Foods are the best sources of calcium; given caloric restriction, the best food sources of calcium are fat free milk and yogurt, and then calcium-fortified foods. Supplements are convenient and often necessary but should not be a substitute for a national nutritional policy or for a good diet. The elderly are commonly vitamin D-deficient as well as calcium-deprived. Conscious efforts must be made to ensure a daily intake of at least 600 to 1000 IU/d. The elderly often suffer some degree of global undernutrition in addition to their specific deficiencies of calcium and vitamin D. Given the common isolation of elderly living alone, this is not an easy problem to solve. At the very least, we must make an effort to feed them after they develop fractures.

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VI Prevention of Major Disabilities: Improvement in Health Outcomes

19 Antioxidant Status and Risk for Cataract

Mark Siegal, Chung-Jung Chiu, and Allen Taylor

KEY POINTS

- Oxidative insult to lens proteins and proteases, together with limited ability to remove the damaged proteins, are involved in the etiology of age-related cataract.
- Observational studies indicate elevated levels of intake of antioxidants is associated with diminished prevalence, incidence, or progress of age-related cataract. They imply that having a daily intake of about 250 mg of vitamin C and 90 mg of vitamin E is associated with diminished risk for cataract. Much of this can be obtained from diet.
- There have been six intervention trails. The results from these are generally inconsistent with the results from the observational trials. It would be worthwhile to determine why the various types of studies are not yielding similar results.
- Most studies indicate associations between poverty, lack of education, lower socioeconomic status, and increased risk for age-related eye diseases. For such persons, a supplement might be indicated, failing remediation of the situation.

1. INTRODUCTION

Oxidative stress is associated with compromises to the lens, including risk for cataract (reviewed in ref. *1*). This chapter briefly describes the etiology of cataract and reviews the epidemiological information regarding nutrition and cataract, with an emphasis on the roles of nutritional antioxidants such as vitamins C and E and carotenoids.

Cataract is one of the major causes of preventable blindness worldwide *(2–4)*. In the United States, the prevalence of visually significant cataract increases from approx 5% at age 65 yr to about 50% for persons older than 75 yr *(5–7)*. In less developed countries, such as India *(8)*, China *(9)*, and Kenya *(10)*, cataracts are more common and develop earlier in life than in more developed countries *(7,8)*. The impact of cataract on impaired vision is much greater in less developed countries, where more than 90% of the cases of blindness and visual impairment are found *(11,12)* and where there is a dearth of ophthalmologists to perform lens extractions. Rates of increase in cataract

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increase as life expectancy extends. About 1.4 million extractions were performed in 1995; this number is expected to double by 2030.

A more optimistic vantage point estimates that a delay in cataract formation of about 10 yr would reduce the prevalence of visually disabling cataract by about 45% *(2)*. Such a delay would enhance the quality of life for much of the world's elderly population and substantially reduce the economic burden (about $5 billion annually in the United States) of cataract-related disability and cataract surgery *(13)*.

1.1. Organization of This Chapter

The chapter begins with a description regarding what we currently know about the etiology of cataract and a summary of the pertinent biochemistry of antioxidants. The available epidemiological data are then described, with particular emphasis on the relations between specific antioxidant nutrients and cataract. Each of these sections begins with a general overview. Decriptions of the relationships between intake of the nutrient and risk for specific forms of cataract follow. A summary of data from recent large intervention trials is a new feature.

2. ETIOLOGY OF CATARACT

The primary function of the eye lens is to collect and focus light on the retina. To perform this function, it must remain clear throughout life (Fig. 1A). The lens is exquisitely organized. A single layer of epithelial cells is located directly under the anterior surface of the collagenous membrane in which it is encapsulated (Fig. 1B). The epithelial cells at the germinative region divide, migrate posteriorly, and differentiate into lens fibers. As their primary gene products, the fibers elaborate the predominant proteins of the lens, called crystallins. New cells form throughout life, but older cells usually are not lost. Instead, they are compressed into the center or nucleus of the lens. With aging or stress caused by light exposure *(14)* or smoking *(15)*, the proteins are photo-oxidatively damaged and aggregate. High-energy radiation, reactive oxygen species, sunlight, and failure of secondary defense systems are among the oxidative insults (*see* Subheadings 2.1. and 2.2.) (Fig. 2). There is a coincident dehydration of the proteins and the lens itself. Consequently, protein concentrations rise to hundreds of milligrams per milliliter *(16)*. Because of age-related modifications of the protein (such as glycation, glycoxidation, modification of proteins by lipid, oxidation of these moieties, and crosslinking), the lens becomes less flexible, with limited accommodative capability. Eventually, the aggregated proteins precipitate in lens opacities, or cataracts.

The term "age-related cataract" distinguishes lens opacification associated with old age from opacification associated with other causes such as congenital and metabolic disorders or trauma. There are several systems for evaluating and grading cataracts. Most of these use an assessment of extent (or density) and location of the opacity *(17,18)*. Opacities are usually evaluated in the posterior subcapsular (PSC), nuclear, cortical, and multiple (mixed) locations; coloration may also be evaluated *(19,20)*. However, it has not been established whether cataract at each location has a completely different etiology.

2.1. Antioxidants as Primary Defenses Against Lens Damage

What systems are available to protect the lens? Protection against photo-oxidative insult can be conceived as being the result of two interrelated processes (Fig. 2). Antioxidants and antioxidant enzymes provide primary protection against insults by

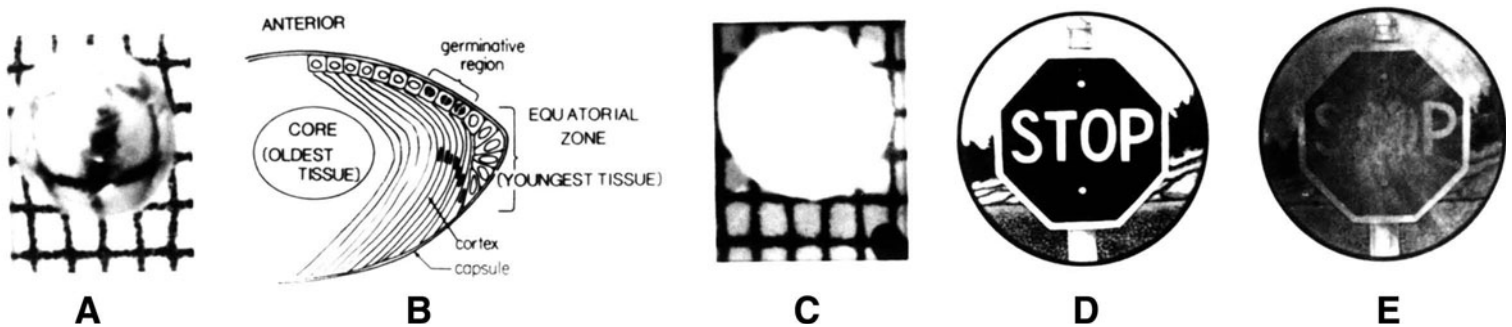

Fig. 1. Clear and cataractous lens. **(A)** Clear lens allows an unobstructed view of the wire grid placed behind it. **(B)** Cartoon of the structure of the lens. The anterior surface of the lens has a unicellular layer of epithelial cells (youngest tissue). Cells at the anterior equatorial region divide and migrate to the cortex as they are overlaid by less mature cells. These cells produce a majority of the crystallins. As development and maturation proceed, the cells denucleate and elongate. Tissue originally found in the embryonic lens is found in the core or nucleus (oldest tissue). **(C)** The cataractous lens prohibits viewing the wire grid behind it. **(D)** Artist's view through a clear, uncolored young lens. The image is clear and crisp. **(E)** Artist's view through a lens with developing cataract. The image is partially obscured and the field is darkened as a result of browning of the lens that accompanies aging.

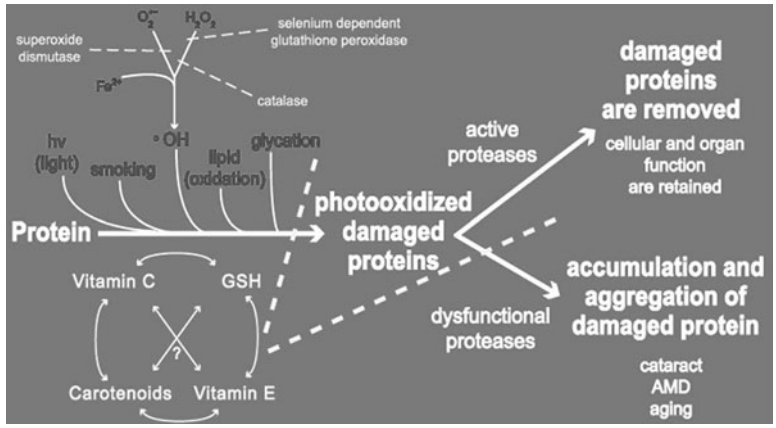

Fig. 2. Proposed interaction between lens proteins, oxidants, light, smoking, antioxidant enzymes, and proteases. Lens proteins are extremely long-lived and subject to alteration by light and various forms of oxygen. They are protected indirectly by antioxidant enzymes: superoxide dismutase, catalase, and GSH reductase/peroxidase. Those enzymes convert active oxygen to less-damaging species. Direct protection is offered by the following antioxidants: GSH, ascorbate (vitamin C), tocopherol (vitamin E), and carotenoids. Levels of reduced and oxidized forms of some, but perhaps not all, of those molecules are determined by an interaction between the three and with the environment *(17–20,23,77)*. In many systems, GSH and ascorbate levels are related, but we did not find this to be the case in ascorbate-sufficient, ascorbate-requiring rats *(24,25)*. When the proteolytic capability is sufficient, obsolete and damaged proteins might be reduced to their constituent amino acids. With aging, some of the eye antioxidant supplies are diminished, antioxidant enzymes are inactivated, and proteases are less active. That action appears to be related to the accumulation, aggregation, and eventual precipitation in cataractous opacities of damaged proteins. GSH, glutathione; hv, light.

attenuating the insult or repairing the protein. Recent research indicates that there is cooperation between the antioxidants, particularly under stress *(21)*. Secondary defenses include proteolytic and repair processes, which degrade and eliminate damaged proteins and other biomolecules in a timely fashion *(22)*.

The major aqueous antioxidants in the lens are ascorbate and glutathione (GSH) *(23–32)*. Both are present in the lens at millimolar concentrations. Ascorbate is probably the most effective and least toxic antioxidant identified in mammalian systems *(23,25,33–36)*. Ocular levels of ascorbate are related to dietary intake in humans and animals that require ascorbate *(35,36)*. Interestingly, the concentration of vitamin C in the lens was increased with dietary supplements beyond levels achieved in persons who already consumed more than two times the Recommended Daily Allowance (RDA; 70 mg/d) for vitamin C *(23,32)*.

Feeding-elevated ascorbate delayed progress of or prevented galactose cataract in guinea pigs *(37,38)* and rats *(39)*, selenite-induced cataracts in rats *(40)*, and lens opacification in GSH-depleted chick embryos *(41)* and delayed ultraviolet-induced protein and protease damage in guinea pig lenses *(42–45)*. Much of the damage that was prevented by increasing ascorbates only twofold was similar to lens protein damage found in cataracts.

Because ascorbate is a carbohydrate, it is biochemically plausible that vitamin C induces damage in the lens in vivo *(46–48)*. However, presently, there are no data to

support this as a medical concern. Although we found that glycohemoglobin levels increased with increasing dietary ascorbate in ascorbate-requiring rats, the rat lenses were not cataractous *(28)*. Furthermore, mice fed 8% of the weight of their diet as ascorbate did not develop cataract *(49)*.

GSH levels in the eye are several-fold greater than the levels found in whole blood and orders of magnitude greater than the concentration observed in the plasma. Similarly to ascorbate, GSH levels also diminish in the older and cataractous lens *(26)*. Preliminary evidence from studies with galactose-induced cataract indicated some advantages in maintaining elevated GSH status in rats *(29)*. However, it is not clear whether feeding GSH is associated with higher ocular levels of this antioxidant *(29)*. In many systems, GSH and ascorbate levels are related. We verified this relationship under stress conditions and when ascorbate levels were limited *(50)* but not in ascorbate-sufficient rats *(28)*.

Tocopherols and carotenoids are lipid-soluble antioxidants *(51,52)* with probable roles in maintaining membrane integrity *(53)* and GSH recycling *(54)*. Concentrations of tocopherol in the whole lens are in the micromolar range, and levels are elevated in younger tissues *(55)*, but lens and dietary levels of tocopherol do not appear to be related *(56)*. Because most of the compound is found in the membranes, the concentrations may be orders of magnitude higher. Other than differential distribution within lenses *(51)*, age-related changes in levels of tocopherol and carotenoids have not been documented. Tocopherol has been reported to be effective in delaying various induced cataracts in animals, including those induced by galactose *(57,58)*, and aminotriazole-induced cataracts in rabbits *(59)*.

Relationships between carotenoids and eye health were reviewed recently *(1,52)*. Levels of β-carotene and lycopene in lenses are extremely low (near vanishing) *(51,55)*. Instead, lutein and zeaxanthin are the major lens and macular carotenoids (present at 11–44 ng/g of wet weight) *(52,60,61)*. Retinol, retinyl ester (21–50 ng/g of wet weight), and tocopherols (1232–2550 ng/g of wet weight) are also present. The carotenoids, such as vitamin A, also are natural lipid-soluble antioxidants *(53,62–64)*. β-carotene is the most well-known carotenoid because of its importance as a vitamin A precursor. Data that relate carotenoid supplement use to risk for cataracts are growing, and carotenoid supplements have already been included in several "eye vitamin" preparations, including the Age-Related Eye Disease Study (AREDS) and the Roche European American Cataract Trial (REACT).

The lens also contains antioxidant enzymes (GSH peroxidase/reductase, catalase, and superoxidase dismutase) and enzymes of the GSH redox cycle *(65–69)*. These interact with the high-energy forms of oxygen and with the antioxidants (GSH is a substrate for GSH peroxidase). The activities of many antioxidant enzymes are compromised with development, aging, and cataract formation *(70)*.

2.2. Proteolytic Enzymes Provide Secondary Defenses Against Lens Damage

The accumulation of damaged proteins is cytotoxic, as has been well-documented in many age-related neurodegenerative diseases including Alzheimer's and Pick's diseases *(71)*. However, nowhere is the cytotoxicity more obvious than in cataract, which is caused by the precipitation of damaged proteins. Proteolytic systems can be considered secondary defense capabilities that remove cytotoxic damaged or obsolete proteins from lenses and other tissues *(22,62,72–76)*. These proteolytic systems exist in younger lens tissue and the outer tissues in all lenses and are probably protected by the primary antioxidants. However, with aging or oxidative stress, most of these enzymatic capabilities are found in a state of reduced activity *(1,77)*. The observed accumulation of oxidized (and/or otherwise modified) proteins in older lenses is consistent with the failure

of these protective systems to keep pace with the insults that damage lens proteins. This occurs partly because, similarly to bulk proteins, enzymes that comprise some of the protective systems are damaged by photo-oxidation *(1,42,74,78–80)*.

Several studies have suggested interactions between antioxidants in which one might spare the other (Fig. 2). Direct sparing effects of ascorbate and GSH on (photo)-oxidatively induced compromises of proteolytic function have been demonstrated *(42,72,74,79)*. From these data, it is clear that the young lens has significant primary and secondary protection. However, age-related compromises in the activity of antioxidant enzymes, concentrations of the antioxidants, and activities of secondary defenses probably lead to diminished protection against oxidative insults. This diminished protection leaves the long-lived proteins and other constituents vulnerable. As the damaged proteins aggregate and precipitate, lens opacities develop. We predicted that elevated antioxidant intake can be exploited to extend the function of some of these proteolytic capabilities, and this might be associated with prolonged lens clarity in vivo *(42)*.

3. EPIDEMIOLOGICAL STUDIES REGARDING ANTIOXIDANTS AND CATARACT

Within the last two decades, considerable effort has been dedicated to determining whether antioxidants can diminish the risk for cataract. The overall impression created by the observational data is that nutrient intake or blood nutrient levels are related to risk for cataract and that nutrition might be exploited to diminish the risk for this debility. The data from the intervention trials are less encouraging upon first inspection. Here, we attempt to summarize both sets of information.

In this chapter, the data regarding a specific nutrient are grouped together. Because of the assumption that the opacities in the PSC, cortical, and nuclear zones of the lens have different etiologies, the data are subdivided to consider cataracts separately for these metabolically and developmentally distinguishable areas . In some studies, the cataract is purely of the type described, whereas in others, additional types of cataract in the lens zone under study or in other lens zones may be present. Future reviews should separate the data from these two designs because there may be some mechanistic similarities between various types of cataract. Data also are considered separately for mixed cataract (involving more than one zone of the lens) and cataract extraction. As compared with our prior reviews, the category “any cataract” has been omitted, with the exception of cases of cataract extraction, because this category obscures potential etiological distinctions between cataract types. Nutrition status was assessed with various questionnaires and/or by measuring blood nutrient levels. Differences in nutrient intake assessment instruments should be considered when the data are considered. Within a cataract type, we discuss the effects of supplements, diet (including supplements), and blood levels.

Comparisons of the data from various studies are not straightforward, because studies differ in design, definitions of cataract, cataract classification schemes, definitions of high and low levels of nutrients, ages of subjects, and stages of the cataracts. Duration of measurement of dietary intake of nutrients and frequency of assessment also can affect the accuracy of these analyses, because cataract develops over many years, and one measure may not provide as accurate an assessment of usual intake as multiple measures over time.

Most studies have been observational, but results from six intervention trials were published recently (*see* Table 1). The following sections describe both types of studies. Assets and limitations of various epidemiological designs were discussed in ref. *81*.

Table 1
Studies Reviewed in This Chapter

Researcher(s)/Name of study	*Year*	*Type of study*	*n*	*Reference*
AREDS	2001	Intervention	4596	*102*
Brown et al.	1999	Prospective	36,644	*103*
Chasan-Taber et al.	1999a	Prospective	73,956	*104*
Chasan-Taber et al.	1999b	Prospective	77,466	*105*
Christen et al.	2003	Intervention	20,968	*107*
Chylack et al.	2002	Intervention	445	*106*
Cumming et al.	2000	Retrospective	2900	*82*
Gale et al.	2001	Retrospective	372	*83*
Hankinson et al.	1992	Prospective	50,828	*108*
Italian-American Cataract Study	1991	Retrospective	1477	*84*
Jacques and Chylack	1991	Retrospective	112	*85*
Jacques et al.	1997	Retrospective	294	*86*
Jacques et al.	2001	Retrospective	478	*87*
Jacques et al.	2004	Prospective	407	*109*
Knekt et al.	1992	Prospective	141	*110*
Kuzniarz et al.	2001	Retrospective	385	*88*
Leske et al.	1991	Retrospective	1380	*89*
Leske et al.	1995	Retrospective	1380	*90*
Leske et al.	1997	Retrospective	4314	*91*
Leske et al.	1998	Prospective	764	*111*
Lyle et al.	1999a	Prospective	1354	*113*
Lyle et al.	1999b	Prospective	252	*112*
Mares-Perlman et al.	1994	Retrospective	1862	*92*
Mares-Perlman et al.	1995a	Retrospective	1919	*93*
Mares-Perlman et al.	1995b	Retrospective	400	*94*
Mares-Perlman et al.	1996	Prospective	3220	*114*
Mares-Perlman et al.	2000	Prospective	2434	*115*
Mohan et al.	1989	Retrospective	1990	*96*
McCarty et al.	1999	Retrospective	3271	*95*
McNeil et al.	2004	Intervention	1193	*116*
Nadalin et al.	1999	Prospective	1111	*117*
Robertson et al.	1989	Retrospective	304	*97*
Rouhiainen et al.	1996	Prospective	410	*118*
Seddon et al.	1994	Prospective	17,744	*119*
Sperduto et al.	1993	Intervention	2141; 3249	*120*
Tavani et al.	1996	Retrospective	913	*98*
Teikari et al.	1998	Intervention	28,934	*121*
Taylor et al.	2002	Retrospective	492	*99*
Valero et al.	2002	Retrospective	677	*100*
Vitale et al.	1993	Retrospective	671	*101*

Some studies were retrospective case-control or cross-sectional studies in which levels of intake of nutrients in patients with cataract were compared with levels of intake in individuals with clear lenses *(82–101)*. Our ability to interpret data from retrospective studies is limited by the concurrent assessment of lens status and nutrient levels. Prior diagnosis of cataract might influence the behavior of cases, including diet and bias reporting of the usual diet.

Table 2
Comparison of Intervention Trials

Study	AREDS (2001) *(102)*; $n = 4629$
Design	A randomized, placebo-controlled, clinical trial
Subjects	Median: 68 yr; range: 55–80 yr
Place	United States
Evaluation of lens opacity	An extension of the Wisconsin System for Subjective Classifying Cataracts from photographs; the area of lens involvement is used to assess the severity of cortical and PSC opacities. Optical density of nuclear opacity is graded against a series of seven standard photographs. Progression of lens opacity (nuclear, cortical, and/or PSC) is defined as follows—nuclear: 1.5-unit increase from baseline on a scale ranging from 0.9 to 6.1; cortical: 10% absolute increase in area from baseline within the central 5-mm diameter circle of the lens; PSC: 5% absolute increase in area from baseline within the central 5-mm diameter circle of the lens. Only relatively advanced cataracts were rated as "lens events." The "lens event rates" were relatively high, ranging from 0.7% for cortical to 21.3% for mixed.
Baseline opacity	Nuclear: <2.0: 37%; 2.0–3.9: 48%; ≥4.0: 15%. Cortical: <0.1: 48%; 0.1–4.9: 41%; ≥5.0: 11%. PSC: <0.1: 90%; 0.1–4.9: 8%; ≥5.0: 2%
Supplement	500 mg of vitamin C, 400 IU of vitamin E, 15 mg of β-carotene, 80 mg of zinc (zinc oxide), 2 mg of copper (cupric oxide). Sixty-six percent of participants took Centrum (containing a RDA amount of vitamins C and E, β-carotene, and zinc). This may have reduced the difference in vitamin concentrations between the treated and placebo groups.
Duration	6.3 yr of AREDS supplement use
Result	No significant protective or adverse effect was found.
Conclusion	Use of a high-dose formulation of vitamins C and E and β-carotene in a relatively well-nourished older adult cohort had no apparent effect on the 7-yr risk of development or progression of age-related nuclear, cortical, or PSC lens opacities or visual acuity loss.
Study	REACT Chylack et al. (2002) *(106)*; $n = 445$
Design	A randomized, placebo-controlled, clinical trial
Subjects	297 randomized from the United States and United Kingdom. Before the start of the study, the UK cohort wa relatively poorer in nutritional status. US age mean: 65 yr, UK age mean: 68 yr; $p = 0.0046$
Evaluation of lens opacity	Standardized digital lens images (CASE 2000 CCD) graded using computer-assisted (objective) image analysis. Cortical: % area opaque (0 to 100); PSC: % area opaque

(Continued)

Table 2 *(Continued)*

	(0 to 100); nuclear dip: ln:sd (10 to 36); scatter density anterior 1 mm and posterior 1 mm: sd (200 to 1000). The primary endpoint was the percentage increase in pixels opaque opaque, used as a continuous variable. More advanced cataract progression was found in the UK cohort than in the US cohort.
Baseline opacity	Nuclear dip (pixel density) mean:16.7; LOCS III nuclear grade mean: 2.4; LOCS III cortical grade mean: 1.8; LOCS III PSC grade mean: 0.58.
Supplement	750 mg vitamin C, 600 IU of vitamin E, 18 mg of β-carotene
Duration	3 yr of supplement use
Result	There was about half as much change in the overall cohort who took the supplement for 3 yr. The protection was significant in the US cohort (which had earlier opacities) and in the overall cohort (US+UK) at 3 yr; at no time was protection significant in the UK cohort alone.
Conclusion	Dietary use of the vitamins C and E and β-carotene for 3 yr produced a small deceleration in progression of age-related mixed cataract.
Study	VECAT, McNeil et al. (2004) *(116)* $n = 1193$
Design	A randomized, placebo-controlled, clinical trial.
Subjects	1193 subjects with early or no cataract, ages 55–80 yr (mean: 65 yr)
Evaluation of lens opacity	The eye with the more advanced opacity; Nidek EAS 1000 (Software version 3.01c; fixed mask); Wilmer lens grading system. Cortical: (integral) 0 (0), 0.5 (1/16), 1 (1/8), 2 (1/4), 3 (1/2), 4 (≥1/2); nuclear: 0–4.9; PSC: multiply the verticaland horizontal dimensions measured by the calibration of the slit beam height. The cutoff points for defining cataract were: cortical: 2.0; nuclear: 2.0; PSC: greater or equal to 1 mm^2. The criteria for cataract progression were: cortical: increase more than 1; nuclear: increase more than 0.5; PSC: increase 1 mm^2 or more.
Baseline opacity	Wilmer grade: cortical: median = 0, mean = 0.5, range: 0–4.0; nuclear: median, mean = 1.5, range: 0.5–4.5. Digital grade: nuclear: mean = 8.015 (95% CI 7.99–8.03).
Supplement	500 IU of vitamin E in soybean oil encapsulated in gelatin.
Duration	4 yr of vitamin E supplement use.
Result	For cortical cataract, the 4-yr cumulative incidence rate was 4.5% among those randomized to vitamin E and 4.8% among those randomized to placebo ($p = 0.87$). For nuclear cataract, the corresponding rates were 12.9 and 12.1% ($p = 0.77$). For PSC cataract, they were 1.7 and 3.5% ($p = 0.08$). For any of these forms of cataract, they were 17.1 and 16.7%, respectively. Progression of cortical cataract was seen in 16.7% of the vitamin E-group and

(Continued)

Table 2 *(Continued)*

	18.4% of the placebo group ($p = 0.76$). Corresponding rates for nuclear cataract were 11.4 and 11.9% ($p = 0.84$), and those of any cataract were 16.5 and 16.7%, respectively. There was no difference in the rate of cataract extraction between the two groups ($p = 0.87$).
Conclusion	Vitamin E given for 4 yr at a dose of 500 IU daily did not reduce the incidence or progression of nuclear, cortical, or posterior subcapsular cataracts. These findings do not support the use of vitamin E to prevent the development or slow the progression of age-related cataracts.
Study	ATBC, Teikari et al. (1998) *(121)* $n = 28{,}934$
Design	A randomized, double-blind, placebo-controlled, 2×2 factorial trial in male smokers.
Subjects	28,934 male smokers ages 50–69 yr.
Evaluation of lens opacity	Cataract extraction, ascertained from the National Hospital Discharge Registry.
Baseline opacity	Does not apply.
Supplement	50 mg of α-tocopherol (vitamin E) per day, 20 mg of β-carotene per day.
Duration	5–8 yr (median: 5.7 yr).
Result	Neither α-tocopherol (RR = 0.91, 95% CI 0.74–1.11) nor β-carotene (RR = 0.97, 95% CI 0.79–1.19) supplementation affected the incidence of cataract surgery.
Conclusion	Supplementation with α-tocopherol or β-carotene does not affect the incidence of cataract extractions among male smokers.
Study	Linxian, Sperduto et al. (1993) *(120)*
Design	Two randomized, double-masked trials with end-of-trial eye examinations.
Subjects	Trial 1: 2141 subjects ages 45–74 yr; Trial 2: 324 subjects ages 45–74 yr.
Evaluation of lens opacity	The Lens Opacities Classification System II, which uses photographic standards for grading cataract type and severity, was used to evaluate lens status at the slit lamp. The system provides ordinal scores for separately grading nuclear (NO, NI, NII, NIII, or NIV), cortical (CO, Ctr, CI CII, CIII, CIV, OR CV), and PSC (PO, PI, PII, PIII, or PIV) opacities. A person was judged to have a nuclear cataract if either eye had a grade of NII or greater, a cortical cataract if either eye had a grade of CII or greater, and a PSC cataract if either eye had a grade of PI or greater. The remaining lens was used to classify persons with unilateral aphakia.
Baseline opacity	Does not apply.

(Continued)

Table 2 *(Continued)*

Supplement	Multivitamin/mineral supplement or matching placebo intrial 1; factorial design to test the effect of four different vitamin/mineral combinations in trial 2 (retinol/zinc, riboflavin/niacin, ascorbic acid/molybdenum, and selenium/ α-tocopherol/β-carotene).
Duration	5–6 yr.
Result	In the first trial, there was a statistically significant 36% reduction in the prevalence of nuclear cataract for persons ages 65–74 yr who received the supplements. In the second trial, the prevalence of nuclear cataract was significantly lower in persons receiving riboflavin/niacin compared with persons not receiving these vitamins. Again, persons in the oldest group (65–74 yr) benefited the most (44% reduction in prevalence). No treatment effect was noted for cortical cataract in either trial. Although the number of PSC cataracts was very small, there was a statistically significant deleterious effect of treatment with riboflavin/niacin.
Conclusion	Increased β-carotene intake and supplement use is not related to diminished risk for nuclear, cortical, or PSC cataract. Increased riboflavin intake or supplement use is related to diminished risk for nuclear cataract. Increased multivitamin supplement use or antioxidant index intake is not related to diminished risk for cortical cataract.
Study	Physician's Health Study, Christen et al. (2003) *(107)* Intervention.
Design	A randomized, double-masked, placebo-controlled trial.
Subjects	Male US physicians ages 40–84 yr, (n = 22,071)
Evaluation of lens opacity	Does not apply.
Baseline opacity	Does not apply.
Supplement	50 mg of β-carotene.
Duration	Alternate days for 12 yr.
Result	Christen et al. found a 27% reduced rate of cataract extraction (RR = 0.73, 95% CI 0.53–1.0) between men who were current smokers who took the β-carotene supplement versus those on a placebo, in a prospective study of 20,968 subjects for 12 yr.
Conclusion	Randomized trial data from a large population of healthy men indicated no overall benefit or harm of 12 yr of β-carotene supplementation on cataract or cataract extraction. However, among current smokers at baseline, β-carotene appeared to attenuate their excess risk of cataract by about one-fourth.

Table 3
Results From Studies That Did Not Use Fixed Endpoints

			Change			
Study	*Design*	*Type of Change*	*Not supplemented*	*Supplemented*	*Difference (not supp –supp)*	*p*
REACT[a], Chylack et al. (2002), *n* = 158	Placebo-controlled intervention trial using daily supp: 18 mg of β-carotene, 750 mg of vitamin C, 600 mg of vitamin E	% Pixels opaque during 3 yr	3.3% Pixels opaque in placebo	1.7% Pixels opaque in in supplement users	1.6%	0.05
NVP, Jacques et al. (2004), *n* = 407	Vitamin E supplement use ≥10 yr	Change in pixels opaque over 5 yr	17	12	5	0.004

[a]A double-masked, randomized, placebo-controlled. Subjects older than age 40 yr had some early cataract at baseline in one eye, cataract was more severe in the United Kingdom than in the United States. Subjects in the United Kingdom had two-thirds lower plasma vitamin C, vitamin E, and lycopene, and one-third lower cryptoxanthin. In the subjects from the United Kingdom, 23% were smokers; in the subjects from the United States, 15% were smokers. The difference in pixels opaque in the United States was 0.0014 after 3 yr; the difference in pixels opaque in the United Kingdom was not significant.

Other studies were prospective and assessed nutrient intake levels and/or supplement use and then followed individuals with intact lenses for up to 13 yr *(102–121)*. In the Nutrition and Vision Project (NVP), we used retrospectively gathered nutrient intake data in a prospective analysis regarding odds for early lens opacities *(87,99)*. Such prospective studies are less prone to bias because assessment of exposure is performed before the outcome is known. Some of those studies *(103–105,108,110,119)* used cataract extraction or reported diagnosis of cataract as a measure of cataract risk. Although risk ratios, which are presented in some studies, are adjusted for many potentially confounding variables, others are not. Data from retrospective and prospective studies are separated in the figures.

Theoretically, the best studies are randomized, double-blind, placebo-controlled, intervention trials. We review six interventions of this type, with follow-ups ranging from 3 to 13 yr *(102,106,107,116,120,121)*. (*See* Section 3.1. and Table 2.)

Most prospective studies used one or more fixed endpoints for their outcome criteria, such as incidence of new cataract, progression to a given opacity gradem or progress of a fixed amount. However, two prospective studies did not use fixed endpoints *(106,109)* (*see* Table 3) but quantified the average change over time in a computer-assisted measurement of opacification.

This chapter features data from studies that found statistically significant correlations between nutrient intake or plasma level of nutrients and risk for opacities. We also distinguish "suggestive associations" between nutrient intake and risk for opacities when

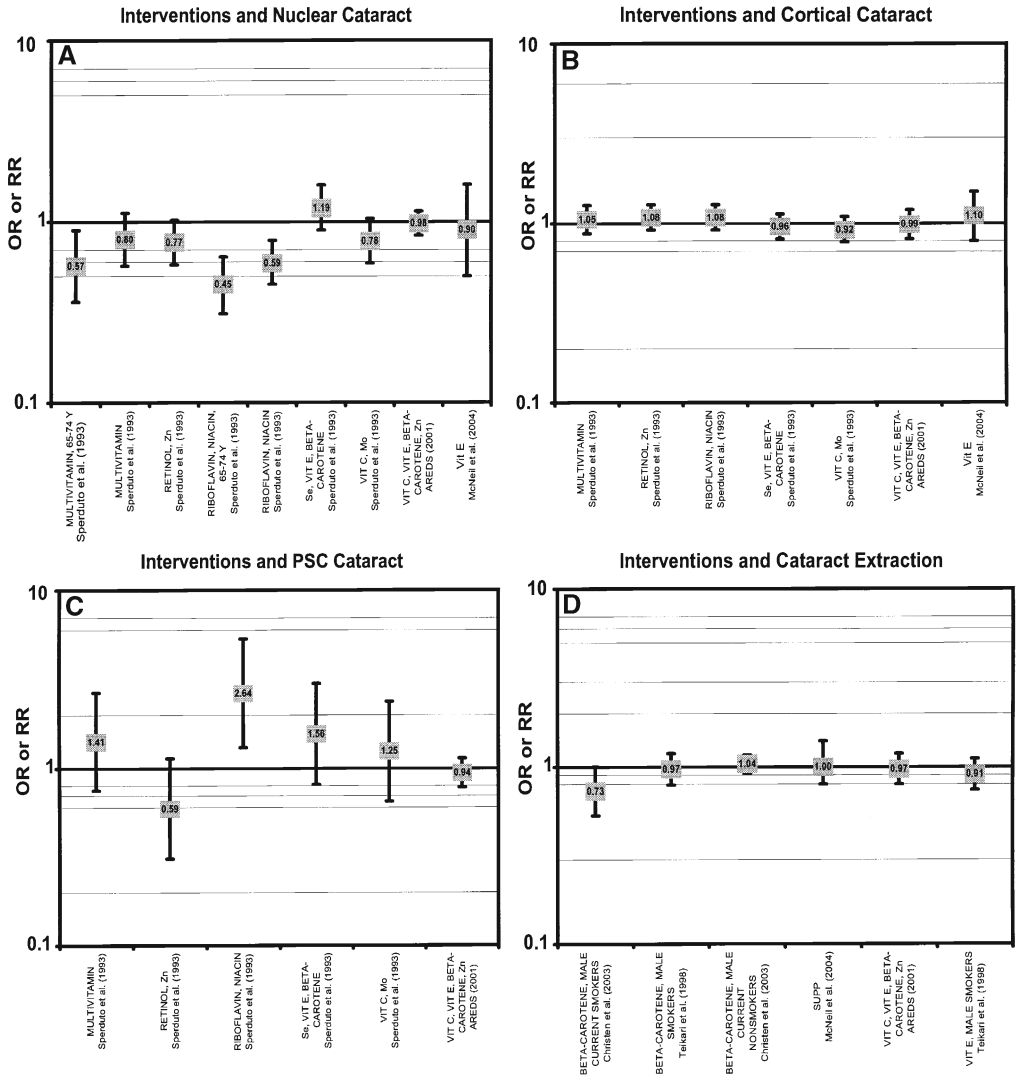

Fig. 3. Intervention studies and cataract risk ratio for intervention supplement group versus placebo group. Log scale. *See also* Table 3 for results of studies without fixed endpoints.

there is a statistically significant *p*-trend but no such individually significant odds ratio (OR) or risk ratio. The other studies were reviewed recently *(1)*. For convenience, all ratios are presented as risk ratios in the text, and only studies that show statistically significant risk or odds ratios are discussed in detail.

3.1. Interventions

Six randomized, double-blinded, placebo-controlled intervention trials assessed the effect of vitamin supplements on cataract risk, with mixed results. The AREDS, Vitamin E, Cataract and Age-related Macular Degeneration (VECAT), and α-Tocopherol β-Carotene (ATBC) trials all found no statistically significant association. The Linxian, Physician's Health Study, and REACT trials each found associations, primarily protective effects, for certain subgroups (Table 2 and Fig. 3).

Although the AREDS design did not support for the conclusion that use of nutrient supplements decreases risk for progress of opacities, it is possible that early intervention with higher doses of vitamins C and E and β-carotene might be the effective strategy if (a) there was not such widespread use of other vitamin supplements, such as Centrum, among the controls, (b) earlier grades of opacities were observed, and (c) more sensitive grading methods were employed *(122)*.

As a result of the use of different cataract assessment methods, different ages, and natural histories of the opacities within each study group at baseline, it is difficult to compare the studies. However, it appears that the chances of finding relationships may be greater if younger age groups are employed in which the cataracts are at early stages—particularly study groups in which supplement use can be evaluated over a long period of time (i.e., 10 yr) prior to significant levels of cataract. A biochemical corollary of finding suggests that, similarly to aggregation of protein (which, in fact, closely describes a cataract), once the process has started, it is difficult to stop. Another corollary is that good eating practices early in life may have health dividends regarding delay of cataract as well as a host of other age-related debilities. These data corroborate observations from the NVP that use of supplements for 10 yr or more relatively early in life (starting before age 50 yr) is required to obtain the benefit of diminishing chances for onset or progress of lens opacities *(87,99,109)*.

3.1.1. Nuclear

Three intervention studies examined risk for nuclear cataract. One study found statistically significant data to support the hypothesis that supplement interventions are related to diminished risk for nuclear cataract *(120)*. The two other studies showed no effect *(102,116)*.

In an intervention study of 2141 subjects, Sperduto et al. *(120)* found a 43% reduced rate of nuclear cataract (risk ratio [RR] = 0.57, 95% confidence interval [CI] 0.36–0.9) between subjects ages 65–74 yr using a multivitamin supplement and those on a placebo. The study also produced a 41% reduced rate of nuclear cataract (RR = 0.59, 95% CI 0.45–0.79) among all subjects on a riboflavin and niacin supplement and a 55% reduced rate (RR = 0.45, 95% CI 0.31–0.64) between subjects ages 65–74 yr on a riboflavin and niacin supplement.

3.1.2. Cortical

None of the three intervention studies that examined risk for cortical cataract showed an effect *(102,116,120)*.

3.1.3. Posterior Subcapsular

Two intervention studies examined risk for PSC cataract. One study found statistically significant data indicating that supplement interventions are related to increased risk for PSC cataract *(120)*.

In an intervention study of 3249 subjects, Sperduto et al. *(120)* found a 64% increased rate of PSC cataract (RR = 2.64, 95% CI 1.31–5.35) between subjects on a riboflavin and niacin intervention and those on a placebo.

3.1.4. Mixed

In an intervention study of 445 subjects examining risk for mixed cataract, statistically significant data were demonstrated to support the hypothesis that vitamins C and E and β-carotene interventions diminished risk for mixed cataract in US subjects but not in the less well-nourished British subjects (Table 3; *106*).

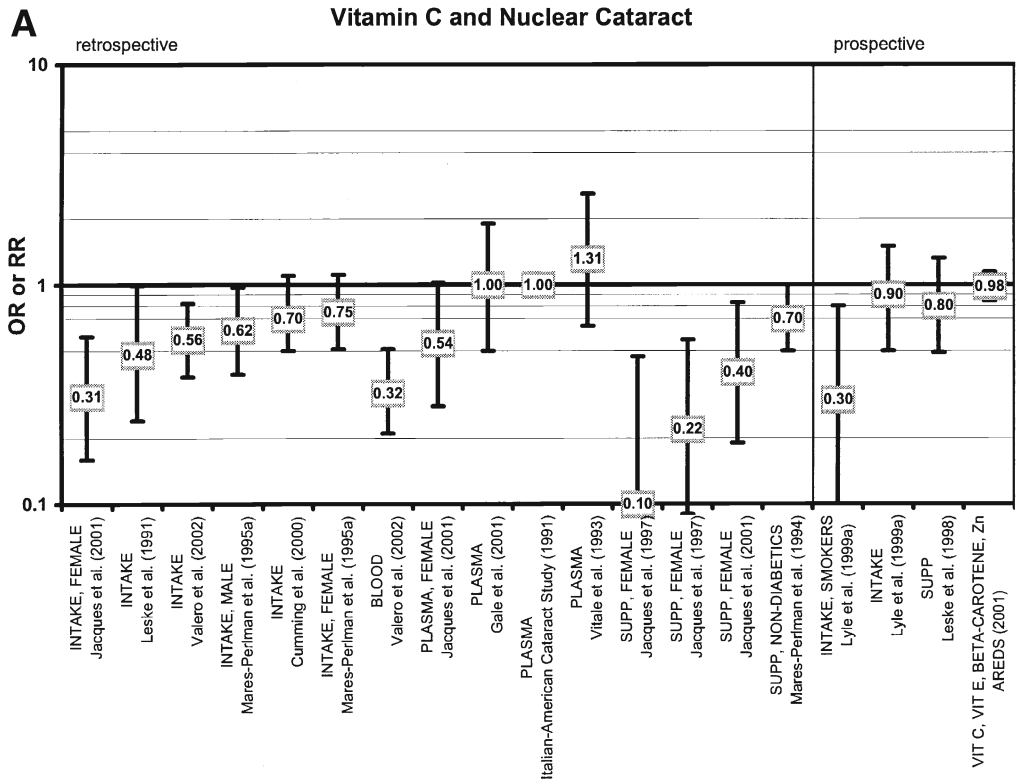

Fig. 4. *(Continued)*

3.1.5. Extraction

Three intervention studies examined risk for cataract extraction. One study found statistically significant data indicating that supplement interventions in male current smokers were related to diminished risk for cataract extraction *(107)*. The other two studies showed no effect *(116,121)*.

In a prospective study of 20968 subjects, Christen et al. *(107)* found a 27% reduced rate of cataract extraction (RR = 0.73, 95% CI 0.53–1.0) between current male smokers on a β-carotene intervention and those on a placebo.

3.2. Observational Studies Regarding Vitamin C (Fig. 4)

The greatest number of studies involved those investigating relations between vitamin C intake and risk for cataract *(82–87,89,92,93,96–102,104,106,108,111,113,119)*.

Biochemical data indicates potential anticataractogenic and cataractogenic roles for vitamin C, but the epidemiological record is far more supportive of the former *(85–87,89,92,93,96,97,99,100,106,108,113)*. In several human epidemiological studies of vitamin C, supplement use and elevated blood levels of vitamin C were inversely associated with at least one type of cataract *(85–87,89,92,93,96,97,99,100,106,108,113)*. Diets rich in fruits and vegetables, which are also rich in antioxidant nutrients, appear to confer diminished risk for nuclear cataract or cataract extraction. The optimal level of vitamin C intake seems to be more than 130 mg; intakes of more than 300 mg provide no added

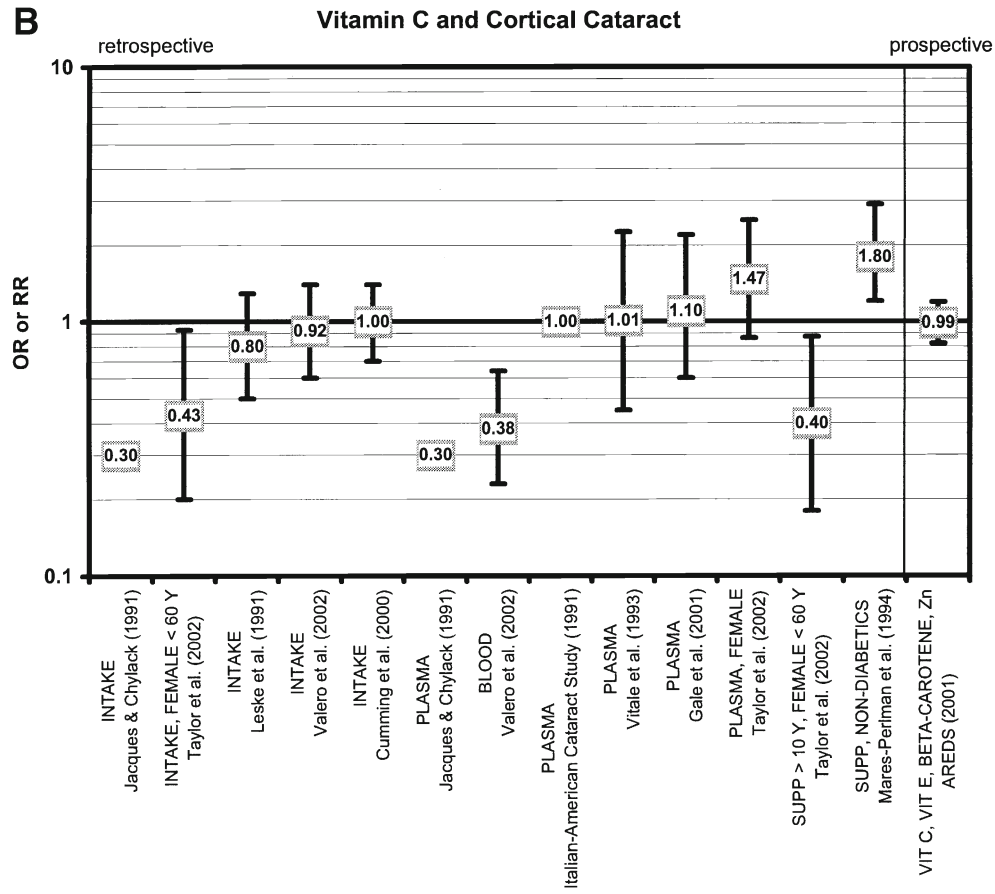

Fig. 4. *(Continued)*

benefit *(87)*. Studies suggest that supplement intake must be maintained for 10 yr to obtain this result *(87,99,115)*.

3.2.1. Nuclear

Thirteen studies examined relations between vitamin C and risk for nuclear cataract. Seven studies found statistically significant data to support the hypothesis that increased vitamin C intake, supplement use, or blood level is related to diminished risk for nuclear cataract *(86,87,89,92,93,100,113)*. The remaining six studies, including the AREDS intervention trial, showed no effect *(82–84,101,102,111)*.

In a retrospective study of 478 subjects, Jacques et al. *(87)* found a 69% reduced rate of nuclear cataract (RR = 0.31, 95% CI 0.16–0.58; p-trend = 0.003) between women with the highest (>362 mg/d) and lowest (<140 mg/d) vitamin C intake. In a retrospective study of 1380 subjects, Leske et al. *(89)* found a 52% reduced rate of cataract (RR = 0.48, 95% CI 0.24–0.99) between subjects with the highest and lowest vitamin C intake. Valero et al. *(100)* found a 44% reduced rate of cataract (RR = 0.56, 95% CI 0.38–0.82) between subjects with the highest and lowest vitamin C intake in a retrospective study of 677 subjects, and Mares-Perlman et al. *(93)* found a 38% reduced rate of cataract (RR = 0.62, 95% CI 0.39–0.97) between men with the highest and lowest vitamin C intake in a retrospective

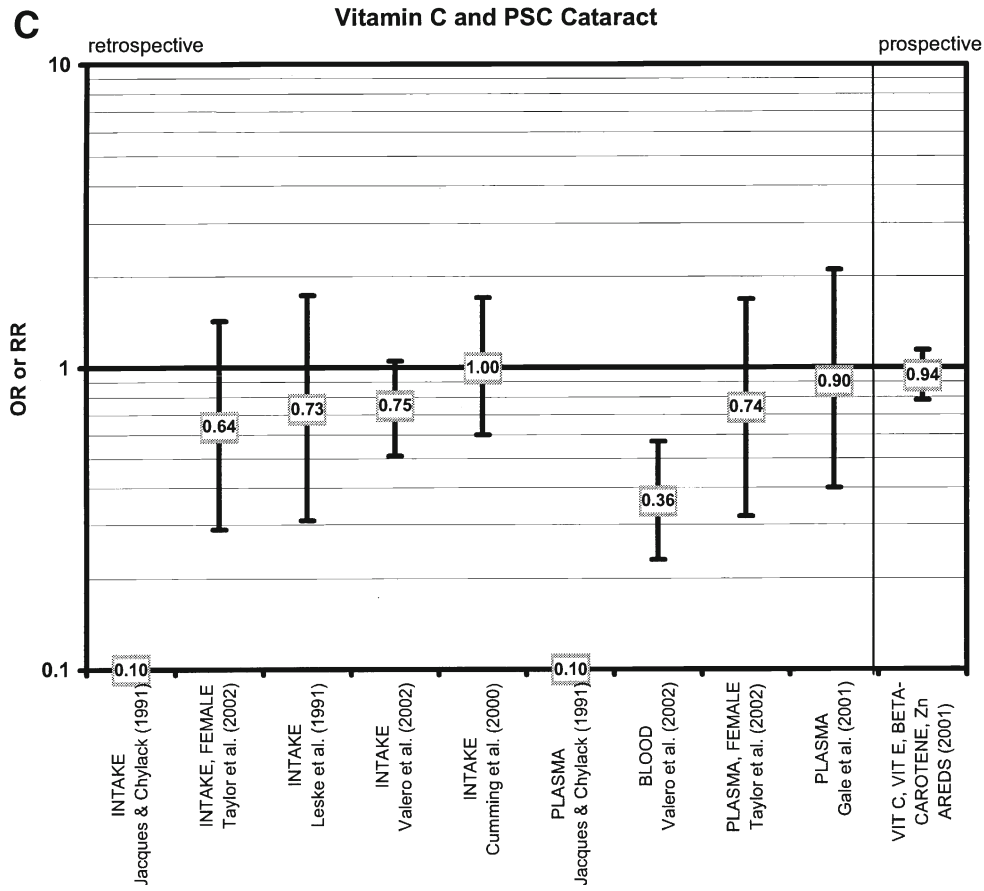

Fig. 4. *(Continued)*

study of 1919 subjects. In a prospective study of 1354 subjects, Lyle et al. *(113)* found a 70% reduced rate of cataract (RR = 0.3, 95% CI 0.1–0.8; *p*-trend = 0.02) between smokers with the highest and lowest vitamin C intake.

The "intake" studies were corroborated by associations between plasma levels of vitamin C and risk for nuclear opacities. The study by Jacques et al. *(87)* was suggestive of a 46% reduced rate of cataract (RR = 0.54, 95% CI 0.28–1.02; *p*-trend = 0.04) between women with the highest and lowest vitamin C plasma. The study by Valero et al. *(100)* demonstrated a 68% reduced rate of cataract (RR = 0.32, 95% CI 0.21–0.51) between subjects with the highest and lowest vitamin C blood.

In the observational studies, vitamin C supplement use often was associated with diminished risk for nuclear opacities. In a retrospective study of 294 subjects, Jacques et al. *(86)* found a 90% reduced rate of moderate cataract (RR = 0.1, 95% CI 0–0.47; *p*-trend = 0.004) and a 78% reduced rate of cataract (RR = 0.22, 95% CI 0.09–0.56; *p*-trend = 0.003) between women who used vitamin C supplements for more than 10 yr vs those who did not. In a more recent study, Jacques et al. *(87)* found a 60% reduced rate of cataract (RR = 0.4, 95% CI 0.19–0.83; *p*-trend = 0.02) between women who used vitamin C supplements for more than 10 yr and those who did not. In a retrospective study of 1862 subjects, Mares-Perlman et al. *(92)* found a 30% reduced rate of cataract (RR = 0.7, 95% CI 0.5–1.0; *p*-trend = 0.002) among nondiabetics who used vitamin C supplements.

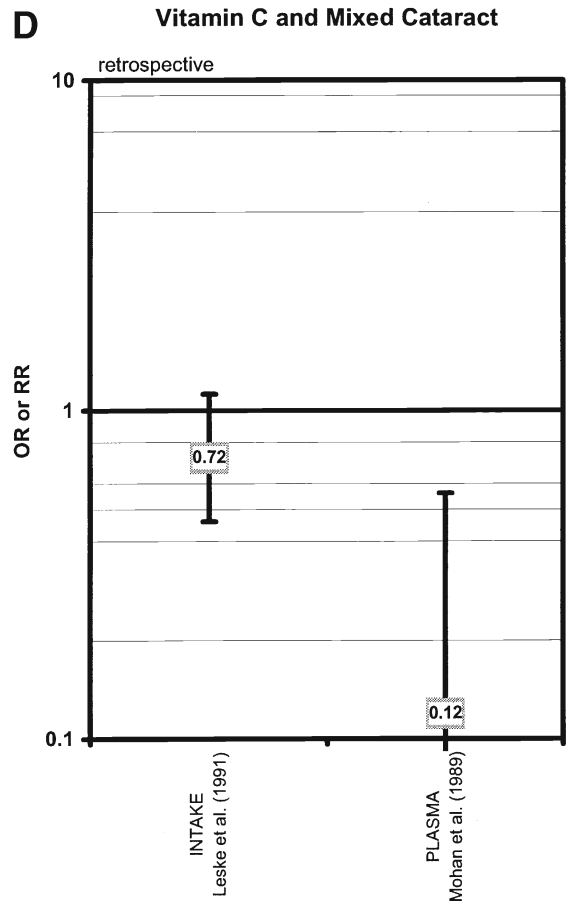

Fig. 4. *(Continued)*

3.2.2. Cortical

Ten studies examined relationships between vitamin C and risk for cortical cataract. Three studies found statistically significant data to support the hypothesis that increased vitamin C intake, supplement use, or blood level is related to diminished risk for cortical cataract *(85, 99,100).* One study found statistically significant data indicating that increased vitamin C supplement use is related to increased risk for cortical cataract *(92)*. The remaining six studies, including the AREDS intervention, showed no effect *(82–84,89,101,102).*

Valero et al. (100) found a 62% reduced rate of cortical cataract (RR = 0.38, 95% CI 0.23–0.64) between subjects with the highest and lowest blood vitamin C. In a retrospective study of 492 subjects, Taylor et al. *(99)* found a 57% reduced rate of cataract (RR = 0.43, 95% CI 0.2–0.93; *p*-trend = 0.02) between women (younger than age 60 yr) with the highest and lowest vitamin C intake and a 60% reduced rate of cataract (RR = 0.4, 95% CI 0.18–0.87; *p*-trend = 0.008) between women (<60 yr) who used vitamin C supplements for more than 10 yr and those who did not.. Mares-Perlman et al. *(92)* found an 80% increased rate of cataract (RR = 1.8, 95% CI 1.2–2.9; *p*-trend = 0.007) in users of vitamin C supplements.

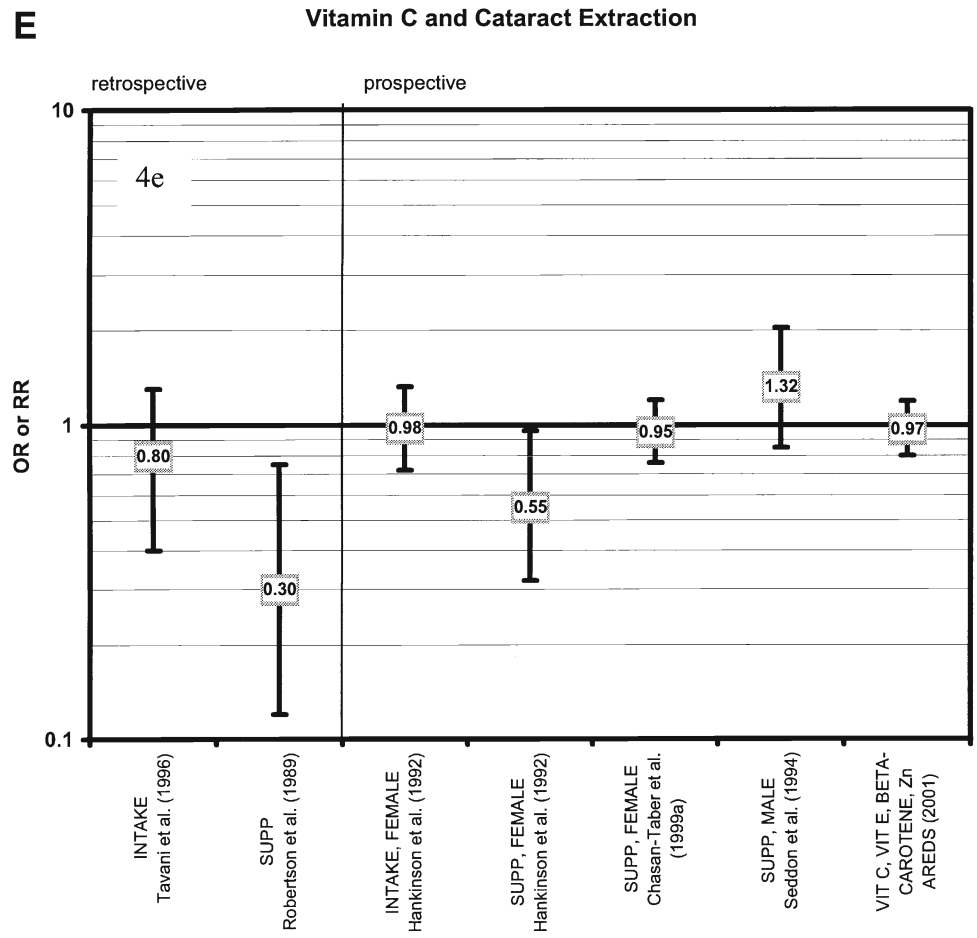

Fig. 4. Vitamin C and cataract risk ratio for high vs low intake (with or without supplements), blood levels, or supplement use. Data for retrospective and prospective studies are presented independently. OR or RR in log scale. *See also* Table 3 for results of studies without fixed endpoints.

3.2.3. Posterior Subcapsular

Seven studies examined relationships between vitamin C and risk for PSC cataract. Two studies found statistically significant data to support the hypothesis that increased vitamin C intake or blood levels is related to diminished risk for PSC cataract *(85,100)*. The remaining five studies, including the AREDS intervention, showed no effect *(82,83,89,99,102)*.

Valero et al. *(100)* found a 64% reduced rate of PSC cataract (RR = 0.36, 95% CI 0.23–0.57) between subjects with the highest and lowest blood vitamin C. In a retrospective study of 112 subjects, Jacques and Chylack *(85)* found a 90% reduced rate of cataract (OR = 0.1) between subjects with the highest and lowest vitamin C intake.

3.2.4. Mixed

Three studies examined relationships between vitamin C and risk for mixed cataract. Two studies, including the REACT intervention, found statistically significant data to support the

hypothesis that increased vitamin C supplement use or blood levels is related to diminished risk for mixed cataract *(96,106)*. In contrast, the study by Leske et al. showed no effect *(89)*.

Mohan et al. *(96)* found an 88% reduced rate of mixed cataract (RR = 0.12, 95% CI 0.03–0.56) between subjects with the highest and lowest plasma vitamin C in a retrospective study of 1990 subjects. (Tables 1 and 2 for REACT intervention data.)

3.2.5. Extraction

Six studies examined relationships between vitamin C and risk for cataract extraction. Two studies found statistically significant data to support the hypothesis that vitamin C supplement use is related to diminished risk for cataract extraction *(97,108)*. The remaining four studies, including the AREDS intervention, showed no effect *(98,102,104,119)*.

In a retrospective study of 304 subjects, Robertson et al. *(97)* found a 70% reduced rate of cataract extraction (RR = 0.3, 95% CI 0.12–0.75) between subjects who did use vitamin C supplements and those who did not.

Hankinson et al. *(108)* found a 45% reduced rate of cataract (RR = 0.55, 95% CI 0.32–0.96) among women who used vitamin C supplements for 10 or more years in a prospective study of 50,828 subjects.

3.3. Observational Studies Regarding Vitamin E

Often, vitamin E supplements provide significantly higher levels of this nutrient than can be obtained in the diet. A variety of studies examined relationships between vitamin E supplement use, dietary intake and plasma levels, and risk for various forms of cataract. The results from the observational studies were mixed (Fig. 5; *109*).

3.3.1. Nuclear

Eighteen studies examined relationships between vitamin E and risk for nuclear cataract. Five studies found statistically significant data to support the hypothesis that increased vitamin E intake, supplement use, or blood level is related to diminished risk for nuclear cataract or cataract progress *(87,90,101,109,111)*. One study found statistically significant data indicating that increased vitamin E blood levels in men are related to increased risk for nuclear cataract *(94)*. Of the remaining studies, 2 were suggestive of decreased risk *(92,93)* and 10, including the AREDS and VECAT interventions, produced no effect *(83,89,95,100,102,112,113,116,117)*.

Jacques et al. *(87)* found a 55% reduced rate of nuclear cataract (RR = 0.45, 95% CI 0.23–0.86) between women with the highest (>90 mg/d) and lowest (<12 mg/d) vitamin E intake. Data from Mares-Perlman et al. *(93)* was suggestive of a 33% reduced rate of cataract (RR = 0.67, 95% CI 0.43–1.03; *p*-trend = 0.03) between men with the highest and lowest vitamin E intakes.

Plasma levels of vitamin E were also related to odds or risk of cataract. Leske et al. *(90)* found a 56% reduced rate of cataract (RR = 0.44, 95% CI 0.21–0.9) between subjects with the highest and lowest plasma level of vitamin E. Jacques et al. *(87)* found a 52% reduced rate of cataract (RR = 0.48, 95% CI 0.25–0.95) between women with the highest and lowest vitamin E plasma level. In a retrospective study of 671 subjects, Vitale et al. *(101)* found a 48% reduced rate of cataract (RR = 0.52, 95% CI 0.27–0.99) between subjects with the highest and lowest plasma level of vitamin E. Mares-Perlman et al. *(94)* found a 3.7-fold increase in the rate of cataract (RR = 3.74, 95% CI 1.25–11.2) between men with the highest and lowest serum vitamin E levels. In a prospective study

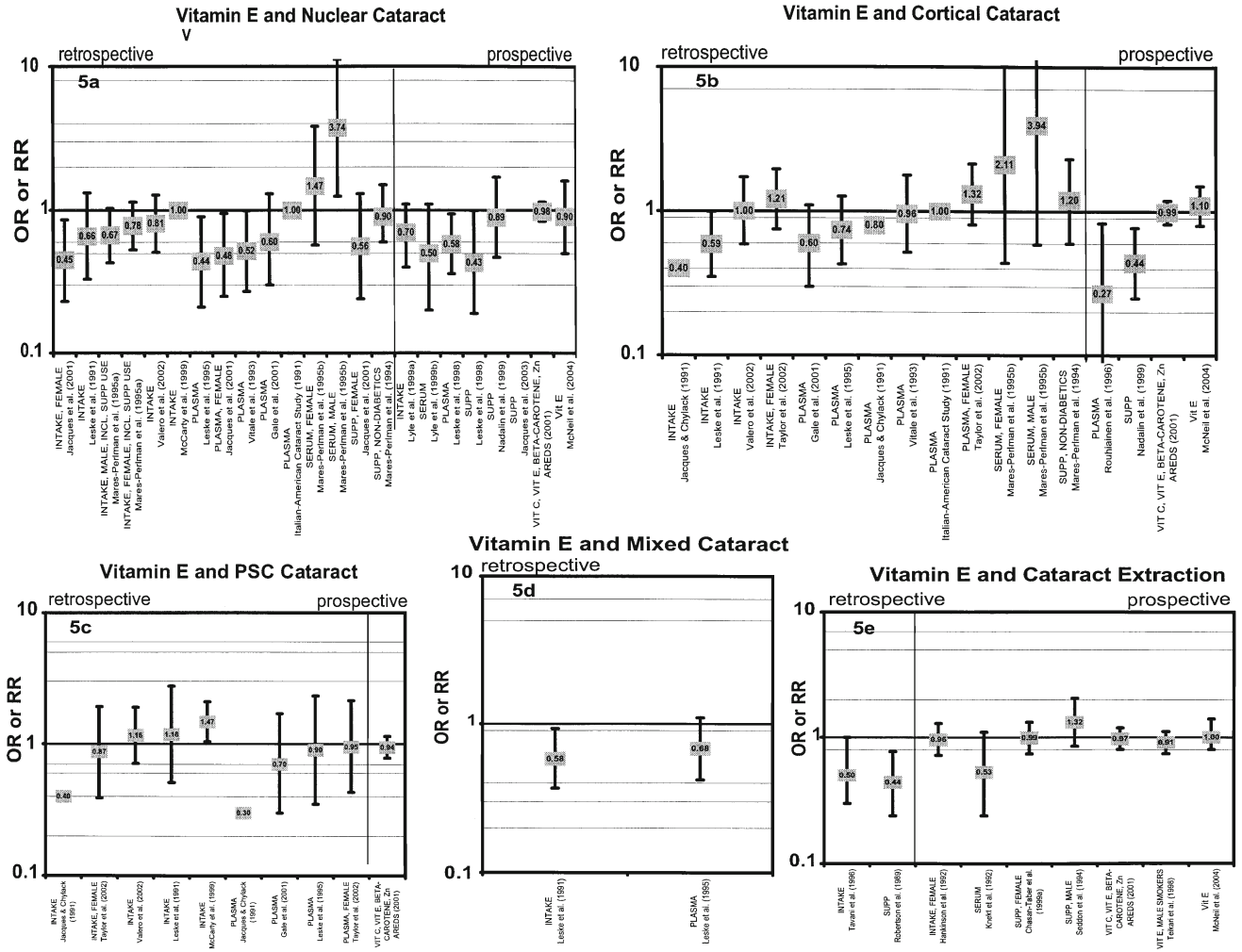

Fig. 5. Vitamin E and cataract risk ratio, for high vs low intake (with or without supplements), blood levels, or supplement use. Data for retrospective and prospective studies are presented independently. OR or RR in log scale. *See also* Table 3 for results of studies without fixed endpoints.

of 764 subjects, Leske et al. *(111)* found a 42% reduced rate of cataract (RR = 0.58, 95% CI 0.36–0.94) between subjects with the highest and lowest plasma levels of vitamin E.

Data from Jacques et al. *(87)* showed a statistically significant trend ($p = 0.03$) for a relationship between the period of use of vitamin E supplements and decreased odds for opacities. In a subsequent study of 40 subjects, we found that use of vitamin E supplements for more than 10 yr is associated with decreased progress of nuclear opacities *(109)*. Supplement use also was related to prevalence or progress of nuclear cataract. The study by Mares-Perlman et al. *(92)* suggested a 10% reduced rate of nuclear cataract (RR = 0.9, 95% CI 0.6–1.5; *p*-trend = 0.02) between users and nonusers of vitamin E supplements. In a prospective study of 764 subjects, Leske et al. *(111)* found a 57% reduced rate of cataract (RR = 0.43, 95% CI 0.19–0.99) between users and nonusers of vitamin E supplements.

3.3.2. Cortical

Fourteen studies examined relationships between vitamin E and risk for cortical cataract. Three studies found statistically significant data to support the hypothesis that increased vitamin E intake, supplement use, or blood level is related to diminished risk for cortical cataract *(89,117,118)*. The remaining 11 studies showed no effect, including the AREDS and VECAT interventions *(83–85,90,92,94,99–102,116)*.

Leske et al. *(89)* found a 41% reduced rate of cortical cataract (RR = 0.59, 95% CI 0.35–0.99) between subjects with the highest and lowest vitamin E intakes. In a prospective study of 410 subjects, Rouhiainen et al. *(118)* found a 73% reduced rate of cataract (RR = 0.27, 95% CI 0.08–0.83) between subjects with the highest and lowest plasma levels of vitamin E. Nadalin et al. *(117)* found a 56% reduced rate of cataract (RR = 0.44, 95% CI 0.25–0.77) between users and nonusers of vitamin E supplements in a prospective study of 1111 subjects.

3.3.3. Posterior Subcapsular

Eight studies examined relationships between vitamin E and risk for PSC cataract. One study of 3271 subjects found statistically significant data indicating that increased vitamin E intake was related to a 47% increased risk for PSC cataract (RR = 1.47, 95% CI 1.04–2.09) *(95)*. The remaining seven studies, including the AREDS intervention, produced no effect *(83,85,89,90,99,100,102)*.

3.3.4. Mixed

Three studies examined relationships between vitamin E and risk for mixed cataract. Two studies (The Lens Opacities Case-Control Study *[89]* and and the REACT intervention *[106]*) found statistically significant data supporting the hypothesis that increased vitamin E intake or supplement use is related to diminished risk for mixed cataract. The remaining study showed no effect *(90)*. (Tables 1 and 2 for REACT intervention data.)

Leske et al. *(89)* found a 42% reduced rate of mixed cataract (RR = 0.58, 95% CI 0.37–0.93) between subjects with the highest and lowest vitamin E intakes. In an intervention study of 445 subjects, Chylack et al. *(106)* found a reduced rate of mixed cataract in US subjects from the REACT intervention but not in the less-than-well-nourished UK subjects (*see* Tables 1 and 2).

3.3.5. Extraction

Nine studies examined relationships between vitamin E and risk for cataract extraction. Two studies found statistically significant data to support the hypothesis that

increased vitamin E intake or supplement use is related to diminished risk for cataract extraction *(97,98)*. The remaining seven studies, including the AREDS, VECAT, and ATBC interventions, showed no effect *(102,104,108,110,116,119,121)*.

In a retrospective study of 913 subjects, Tavani et al. *(98)* found a 50% reduced rate of cataract extraction (RR = 0.5, 95% CI 0.3–1.0) between subjects with the highest and lowest vitamin E intakes. Robertson et al. *(97)* found a 56% reduced rate of cataract extraction (RR = 0.44, 95% CI 0.24–0.77) between subjects who used vitamin E supplements and those who did not in a retrospective study of 304 subjects.

3.4. Observational Studies Regarding Carotenoids

The major carotenoids found in the lens and those that have been related to lens health are lutein, zeaxanthin, and riboflavin. Levels of β-carotene are extremely low (near vanishing). Lutein and zeaxanthin are important carotenoid components of the human diet, and several investigations have suggested that elevated intake of foods rich in lutein, such as spinach, is related to decreased risk for cataract. Results are mixed for lutein and zeaxanthin as well as for other carotenoids (Figs. 6–9).

3.4.1. Total Carotenoids (Fig. 6)

3.4.1.1. Nuclear. Three studies examined relationships between total carotenoids and risk for nuclear cataract. One study *(94)* found statistically significant data indicating that increased serum levels of total carotenoids are related to a 2.95-fold increased rate of cataract in women (RR = 3.95, 95% CI 1.65–9.47) but, curiously, to a 45% risk in men (RR = 0.55, 95% CI 0.2–1.49; *p*-trend = 0.002) . The two remaining studies showed no effect *(87,105)*.

3.4.1.2. Cortical. Three studies examined relationships between total carotenoids and risk for cortical cataract. One study found statistically significant data to support the hypothesis that an increase in total carotenoids blood levels is related to diminished risk for cortical cataract *(85)*. Of the remaining studies, one was suggestive of an increased risk with blood levels in women *(94)* and the other produced no effect *(99)*. Therefore, Jacques and Chylack *(85)* found significantly higher odds (OR = 7.2) of cortical cataract among subjects with the lowest plasma or caratenoid level compared to the highest levels, whereas, Mares-Perlman et al. *(94)* indicated a 3.3-fold increase in the rate of cataract (RR = 3.26, 95% CI 0.92–11.5; *p*-trend = 0.05) in women with the highest vs those with the lowest serum level of carotenoids.

3.4.1.3. Posterior Subcapsular. All three studies that examined relationships between total carotenoids and risk for PSC cataract found statistically significant data supporting the hypothesis that an increase in total carotenoids blood levels or intake is related to diminished risk for PSC cataract *(85,99,105)*.

Taylor et al. *(99)* found an 81% reduced rate of PSC cataract (RR = 0.19, 95% CI 0.05–0.68; *p*-trend = 0.01) in women who had never smoked and who had the highest vs lowest intake of carotenoids. We corroborated this data with an observation of a 59% reduced rate of cataract (RR = 0.41, 95% CI 0.17–0.99) in women with the highest vs lowest plasma level of carotenoids. In a prospective study of 77,466 subjects, Chasan-Taber et al. *(105)* showed a 31% reduced rate of cataract (RR = 0.69, 95% CI 0.49–0.98) between women with the highest and lowest carotenoids intakes.

3.4.1.4. Extraction. Four studies examined relationships between total carotenoids and risk for cataract extraction. Two studies documented statistically significant data to

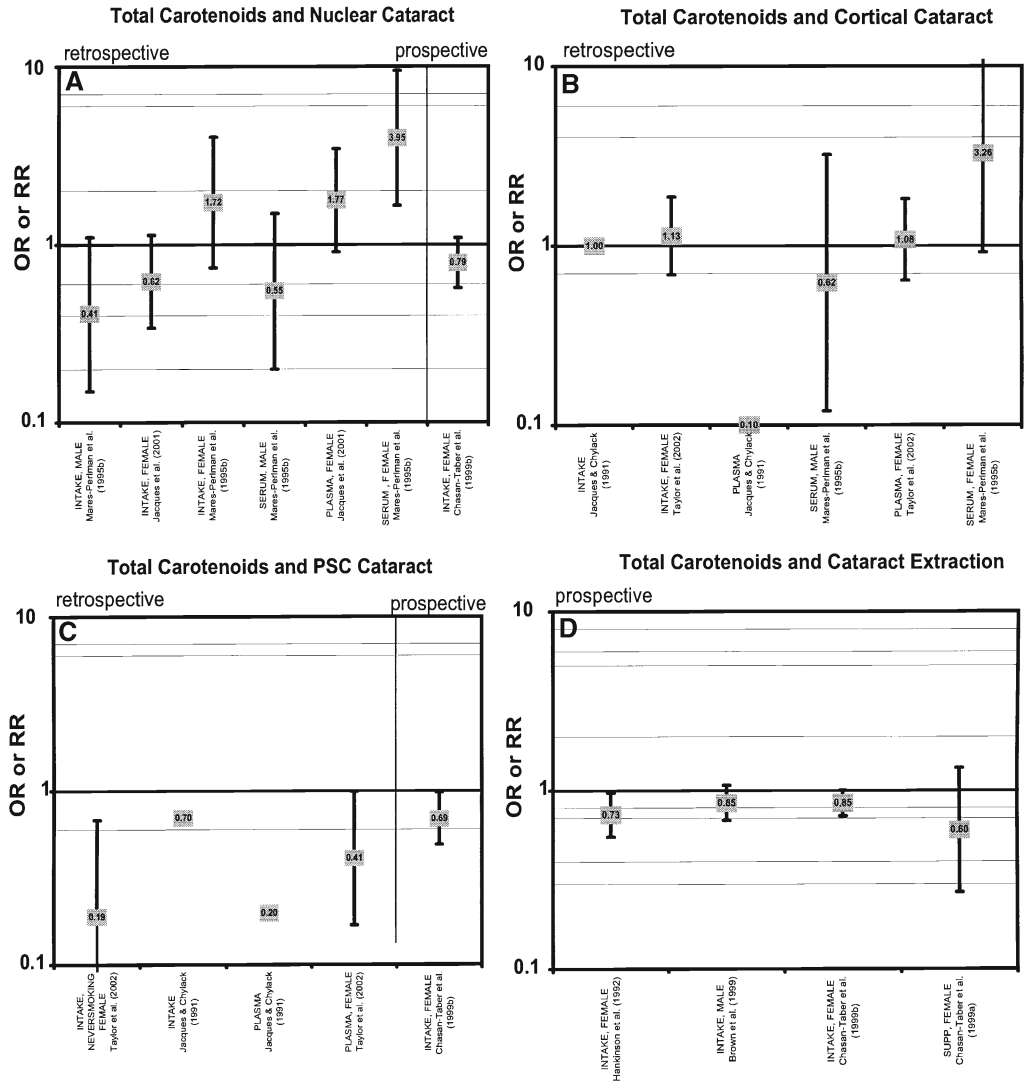

Fig. 6. Total carotenoids and cataract risk ratio for high vs low intake (with or without supplements), blood levels, or supplement use. Data for retrospective and prospective studies are presented independently. OR or RR in log scale.

support the hypothesis that increased total carotenoid intake is related to diminished risk for cataract extraction *(105, 108)*. One study suggested a decreased risk with intake in men *(103)*, and the other produced no effect *(104)*.

Hankinson et al. *(108)* found a 27% reduced rate of cataract extraction (RR = 0.73, 95% CI 0.55–0.97) between women with the highest and lowest intakes of carotenoids in a prospective study of 50,828 subjects. In a prospective study of 77,466 subjects, Chasan-Taber et al. *(105)* found a 15% reduced rate of extraction (RR = 0.85, 95% CI 0.72–1.0) between women with the highest and lowest intakes of carotenoids. A prospective study of 36,644 subjects by Brown et al. *(103)* suggested a 15% reduced

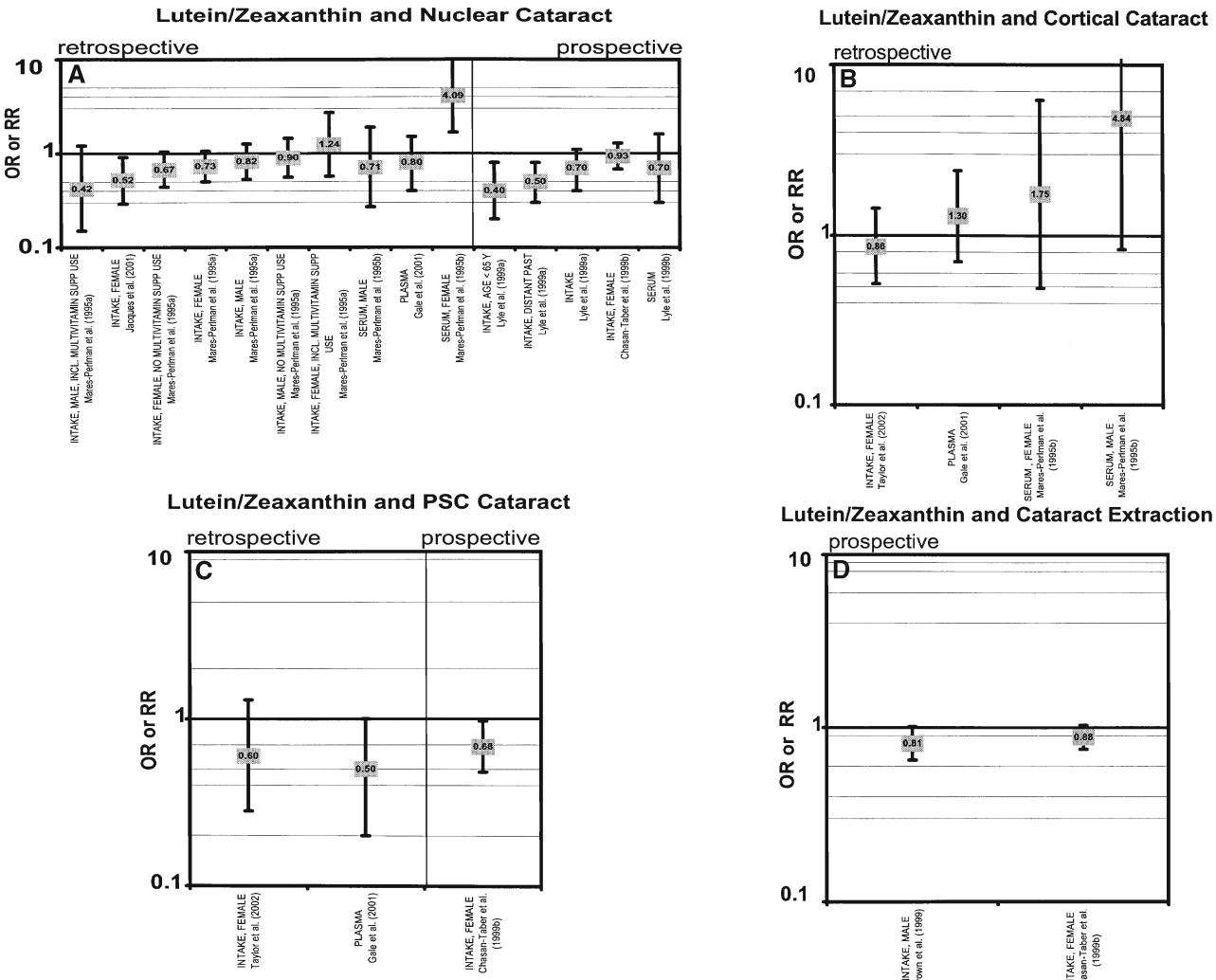

Fig. 7. Lutein/zeaxanthin and cataract risk ratio for high vs low intake (with or without supplements), blood levels, or supplement use. Data for retrospective and prospective studies are presented independently. OR or RR in log scale.

rate of extraction (RR = 0.85, 95% CI 0.68–1.07; *p*-trend = 0.05) between men with the highest and lowest intakes of carotenoids.

3.4.2. Lutein and Zeaxanthin (Fig. 7)

3.4.2.1. Nuclear. Seven studies examined relationships between lutein/zeaxanthin and risk for nuclear cataract. Two studies found statistically significant data supporting the hypothesis that increased lutein/zeaxanthin intake is related to diminished risk for nuclear cataract *(87,113)*. One study found statistically significant indicating that increased lutein/zeaxanthin blood levels in women increase the risk for nuclear cataract *(94)*. One study suggested a decreased risk of nuclear cataract for increased intake of lutein/zeaxanthin in women *(93)*. The remaining three studies produced no effect *(83,105,112)*.

Jacques et al. *(87)* found a 48% reduced rate of nuclear cataract (RR = 0.52, 95% CI 0.29–0.91) between women with the highest and lowest lutein/zeaxanthin intakes. In a prospective study of 1354 subjects, Lyle et al. *(113)* found a 60% reduced rate of cataract (RR = 0.4, 95% CI 0.2–0.8) between subjects (ages <65 yr) with the highest and lowest lutein intakes. A retrospective study of 1919 subjects by Mares-Perlman et al. *(93)* suggested a 27% reduced rate of nuclear cataract (RR = 0.73, 95% CI 0.5–1.06; *p*-trend = 0.02) between women with the highest and lowest lutein intakes. In contrast, in a retrospective study of 400 subjects, Mares-Perlman et al. *(94)* found a fourfold increased rate of nuclear cataract (RR = 4.09, 95% CI 1.67–10.03; *p*-trend = 0.006) between women with the highest and lowest serum levels of lutein. Finally, Lyle et al. *(113)* found that persons in the highest quintile of lutein intake in the distant past were half as likely to have an incident of cataract as persons in the lowest quintile of intake in a prospective study of 1354 subjects (RR = 0.5, 95% CI 0.3–0.8, *p*-trend = 0.002).

3.4.2.2. Cortical. Three studies examined relationships between lutein/zeaxanthin blood levels or intake and cortical cataract. Two studies produced no effect *(83,99)*, whereas the study by Mares-Perlman et al. *(94)* suggested a 4.8-fold increased rate of cortical cataract (RR = 4.84, 95% CI 0.83–28.1; *p*-trend = 0.03) between men with the highest and lowest lutein serum levels.

3.4.2.3. Posterior Subcapsular. Three studies examined relationships between lutein/zeaxanthin and risk for PSC cataract. Two studies found statistically significant data supporting the hypothesis that increased lutein/zeaxanthin intake or blood levels was related to diminished risk for PSC cataract *(83,105)*. The remaining study produced no effect *(99)*.

Gale et al. *(83)* found a 50% reduced rate of PSC cataract (RR = 0.5, 95% CI 0.2–1; *p*-trend = 0.012) between subjects with the highest and lowest plasma levels of lutein in a retrospective study of 372 subjects. Chasan-Taber et al. *(105)* found a 32% reduced rate of cataract (RR = 0.68, 95% CI 0.48–0.97) between women with the highest and lowest intakes of lutein/zeaxanthin.

3.4.2.4. Extraction. Two studies examined relationships between lutein/zeaxanthin intake and risk for cataract extraction. Data from a prospective study of 36,644 subjects by Brown et al. *(103)* suggested a 19% reduced rate of extraction (RR = 0.81, 95% CI 0.65–1.01; *p*-trend = 0.03) between men with the highest and lowest intakes of lutein + zeaxanthin. The other study showed no effect *(105)*.

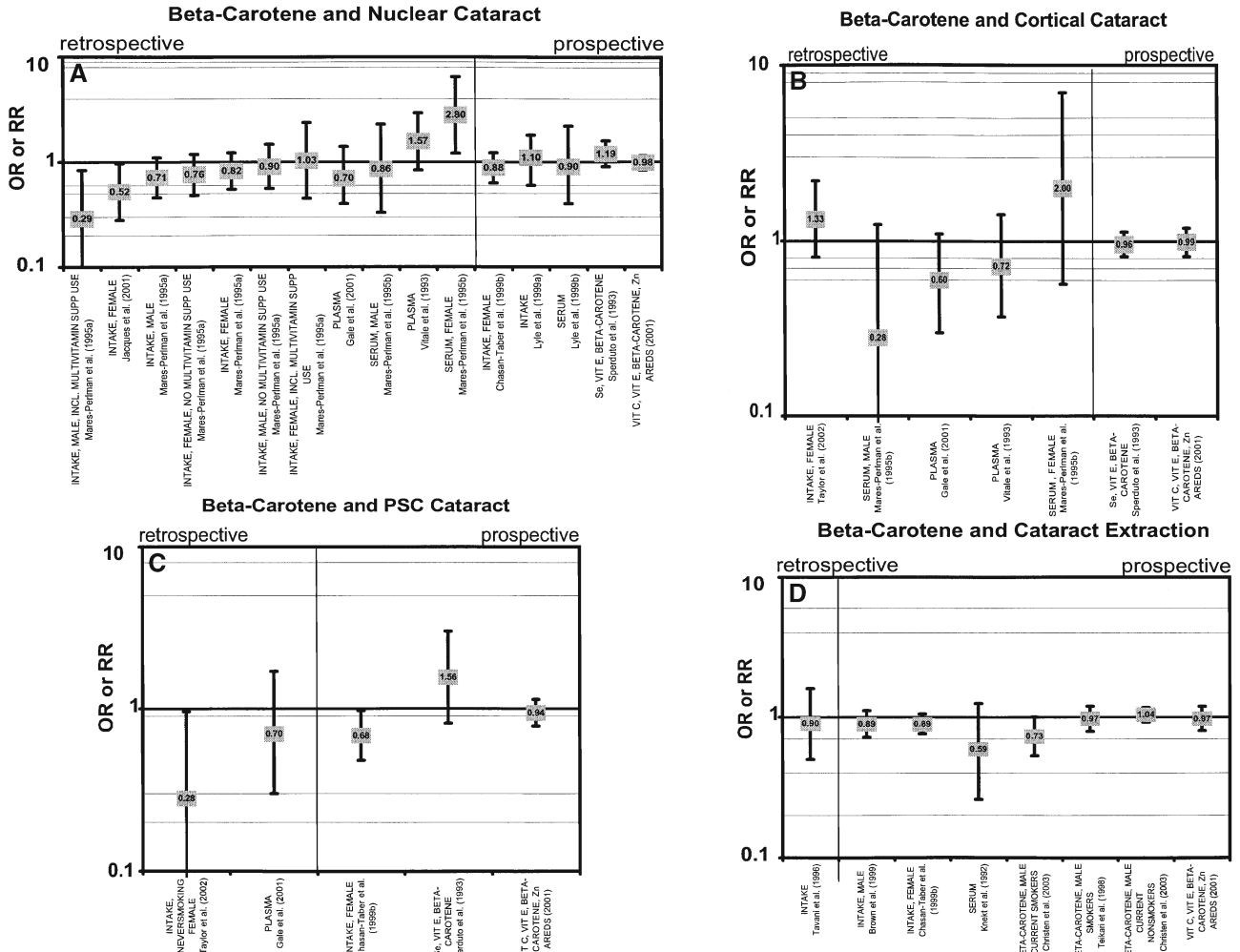

Fig. 8. β-carotene and cataract risk ratio for high vs low intake (with or without supplements), blood levels, or supplement use. Data for retrospective and prospective studies are presented independently. OR or RR in log scale. *See also* Table 3 for results of studies without fixed endpoints.

3.4.3. β-Carotene (Fig. 8)

3.4.3.1. Nuclear. Of the 10 studies that examined relationships between β-carotene and risk for nuclear cataract, 2 found statistically significant data supporting the hypothesis that increased β-carotene intake or supplement use is related to diminished risk for nuclear cataract *(87,93)*, and 1 study found statistically significant data indicating that an increase in β-carotene blood levels is related to increased risk for nuclear cataract *(94)*. Of the remaining studies, one was suggestive of diminished risk *(83)* and the others, including the AREDS and Linxian interventions, showed no effect *(101,102,105,112,113,120)*.

Mares-Perlman et al. *(93)* found a 71% reduced rate of cataract (RR = 0.29, 95% CI 0.1–0.84) between men with the highest and lowest intakes of β-carotene, and we *(87)* demonstrated a 48% reduced rate of cataract (RR = 0.52, 95% CI 0.28–0.97) between women with the highest and lowest intakes of β-carotene.

Data from Gale et al. *(83)* suggested a 30% reduced rate of cataract (RR = 0.7, 95% CI 0.4–1.4; *p*-trend = 0.033) between subjects with the highest and lowest plasma levels of β-carotene. In contrast, Mares-Perlman et al. *(94)* found an 80% increased rate of cataract (RR = 2.8, 95% CI 1.21–6.48) between women with the highest and lowest serum levels of β-carotene.

3.4.3.2. Cortical. Six studies examined relationships between β-carotene and risk for cortical cataract. Data from Mares-Perlman et al. *(94)* suggested a 72% reduced rate of cataract (RR = 0.28, 95% CI 0.06–1.24; *p*-trend = 0.05) between men with the highest and lowest serum levels of β-carotene. The remaining five studies, including the AREDS and Linxian interventions, produced no effect *(83,99,101,102,120)*.

3.4.3.3. Posterior Subcapsular. Five studies examined relationships between β-carotene and risk for PSC cataract. Taylor et al. *(99)* found a 72% reduced rate of cataract (RR = 0.28, 95% CI 0.08–0.96; *p*-trend = 0.02) between women who had never smoked who had the highest and lowest intakes of β-carotene. The remaining studies, including the AREDS and Linxian interventions, produced no effects *(83,102,120)*. In a prospective study of 77,466 subjects, Chasan-Taber et al. *(105)* found a 32% reduced rate of cataract (RR = 0.68, 95% CI 0.48–0.97) between women with the highest and lowest intakes of β-carotene,.

3.4.3.4. Mixed. Only one study that examined relationships between β-carotene and risk for mixed cataract produced significant results. In an intervention study of 445 subjects, Chylack et al. *(106)* found statistically significant data supporting the hypothesis that β-carotene interventions (along with vitamins C and E) diminished risk for mixed cataract in US subjects but not in less well-nourished UK subjects (Table 3).

3.4.3.5. Extraction. Six studies examined relationships between β-carotene and risk for cataract extraction. In a prospective study of 20,968 physicians, Christen et al. *(107)* found a 27% reduced rate of extraction (RR = 0.73, 95% CI 0.53–1.0) between current male smokers who did and did not take the 50-mg β-carotene supplement on alternate days. The five remaining studies, including the AREDS and ATBC interventions, showed no effect *(98,102,103,105,110,121)*.

3.4.4. α-Carotene (Fig. 9)

3.4.4.1. Nuclear. Seven studies examined relationships between α-carotene and risk for nuclear cataract. Four studies showed no effect *(87,105,112,113)*. Mares-Perlman et al. found a 39% reduced rate of cataract (RR = 0.61, 95% CI 0.39–0.95; *p*-trend = 0.04)

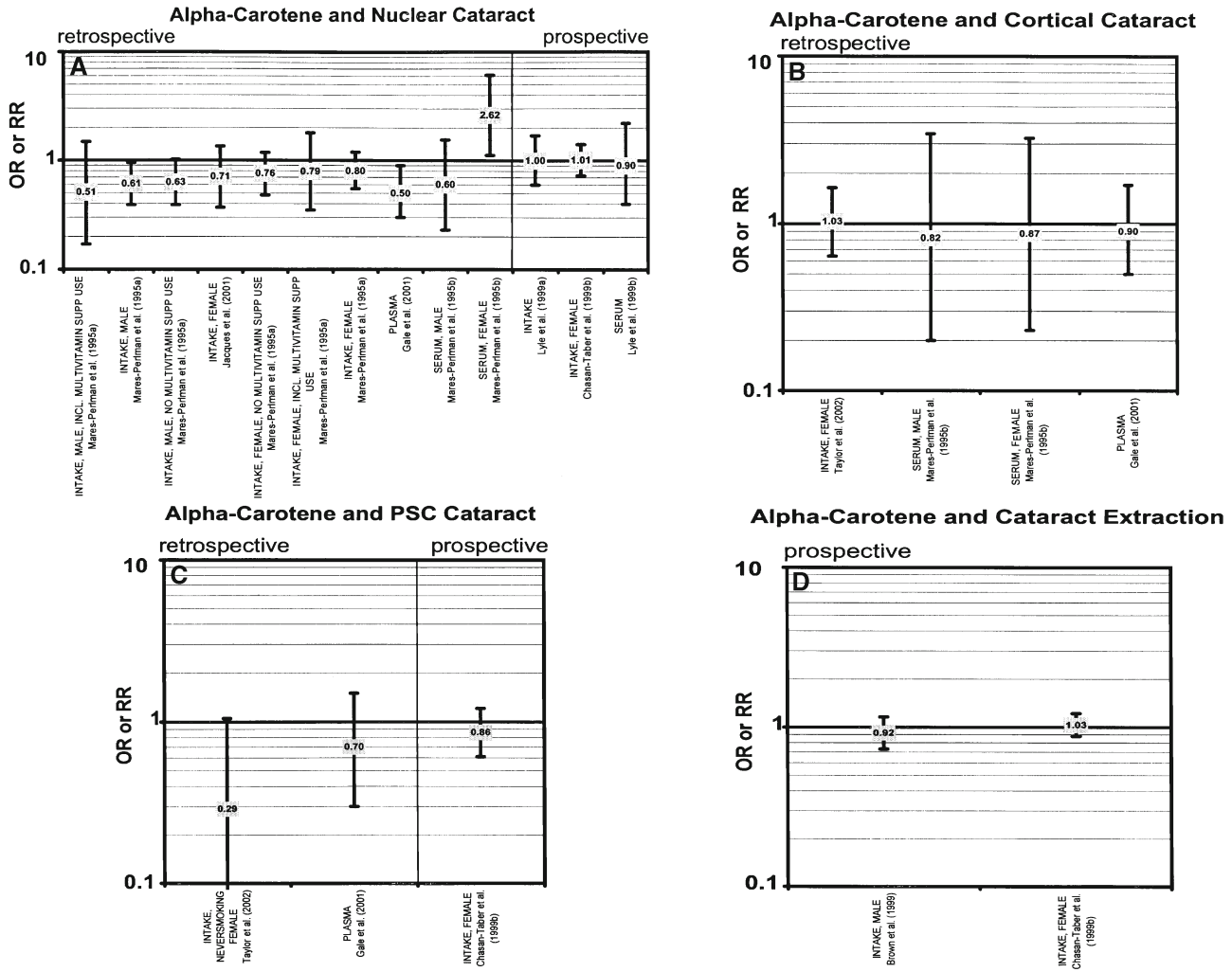

Fig. 9. α-carotene and cataract risk ratio for high vs low intake (with or without supplements), blood levels, or supplement use. Data for retrospective and prospective studies are presented independently. Or or RR in log scale.

between men with the highest and lowest intakes of α-carotene *(93)* but found a 2.62-fold increase in the rate of cataract (RR = 2.62, 95% CI 1.12–6.13) between women with the highest and lowest serum levels of α-carotene in a retrospective study of 400 subjects *(94)*. In contrast, Gale et al. *(83)* found a 50% reduced rate of cataract (RR = 0.5, 95% CI 0.3–0.9; *p*-trend = 0.006) between subjects with the highest and lowest plasma levels of α-carotene.

3.4.4.2. Cortical. None of the three studies that examined relationships between α-carotene and risk for cortical cataract found statistically significant data *(83,94,99)*.

3.4.4.3. Posterior Subcapsular. Three studies examined relationships between α-carotene and risk for PSC cataract. Taylor et al. *(99)* obtained data that suggested a 71% reduced rate of cataract (RR = 0.29, 95% CI 0.08–1.05; *p*-trend = 0.02) between the highest and lowest intakes of α-carotene in women who had never smoked. The remaining two studies found no effect *(83, 105)*.

3.4.4.4. Extraction. Neither of the two studies that examined relationships between α-carotene intake and risk for cataract extraction found statistically significant data *(103,105)*.

3.4.5. Riboflavin (Fig. 10)

3.4.5.1. Nuclear. Seven studies examined relationships between riboflavin and risk for nuclear cataract. Four studies, including the Linxian intervention, found statistically significant data supporting the hypothesis that increased riboflavin intake or supplement use is related to diminished risk for nuclear cataract *(82,87,93,120)*. One study suggested a decreased risk in nondiabetic subjects *(92)*. The two remaining studies found no effect *(88,89)*.

Jacques et al. *(87)* found a 63% reduced rate of cataract (RR = 0.37, 95% CI 0.19–0.73; *p*-trend = 0.03) between women with the highest and lowest intakes of riboflavin. Similarly, Cumming et al. *(82)* found a 50% reduced rate of cataract (RR = 0.5, 95% CI 0.3–0.9; *p*-trend = 0.01) between subjects with the highest and lowest intakes of riboflavin, and Mares-Perlman et al. *(93)* found a 44% reduced rate of cataract (RR = 0.56, 95% CI 0.36–0.87; *p*-trend = 0.009) between men with the highest and lowest intakes of riboflavin.

Mares-Perlman et al. *(93)* also found a 33% reduced rate of cataract (RR = 0.67, 95% CI 0.46–0.98; *p*-trend = 0.026) between women with the highest and lowest intakes of riboflavin. Another study by Mares-Perlman et al. *(92)* suggested a 20% reduced rate of cataract (RR = 0.8, 95% CI 0.5–1.3; *p*-trend = 0.02) in nondiabetics with the highest and lowest riboflavin supplements.

Sperduto et al. *(120)* found a 55% reduced rate of nucleic cataract (RR = 0.45, 95% CI 0.31–0.64) in 65- to 74-yr-old poorly nourished Chinese subjects who took supplements containing riboflavin and niacin. In all subjects of this portion of the Linxian study, there was a 41% reduction in risk for cataract (RR = 0.59, 95% CI 0.45–0.79) in users of this supplement.

3.4.5.2. Cortical. Five studies examined relationships between riboflavin and risk for cortical cataract. Two studies, including the Linxian intervention, found no effect *(99, 120)*. Leske et al. *(89)* found a 41% reduced rate of cataract (RR = 0.59, 95% CI 0.36–0.97) between subjects with the highest and lowest intakes of riboflavin, and Cumming et al. *(82)* found a 30% reduced rate of cataract (RR = 0.7, 95% CI 0.5–1.0) between subjects with the highest and lowest intakes of riboflavin. However, data

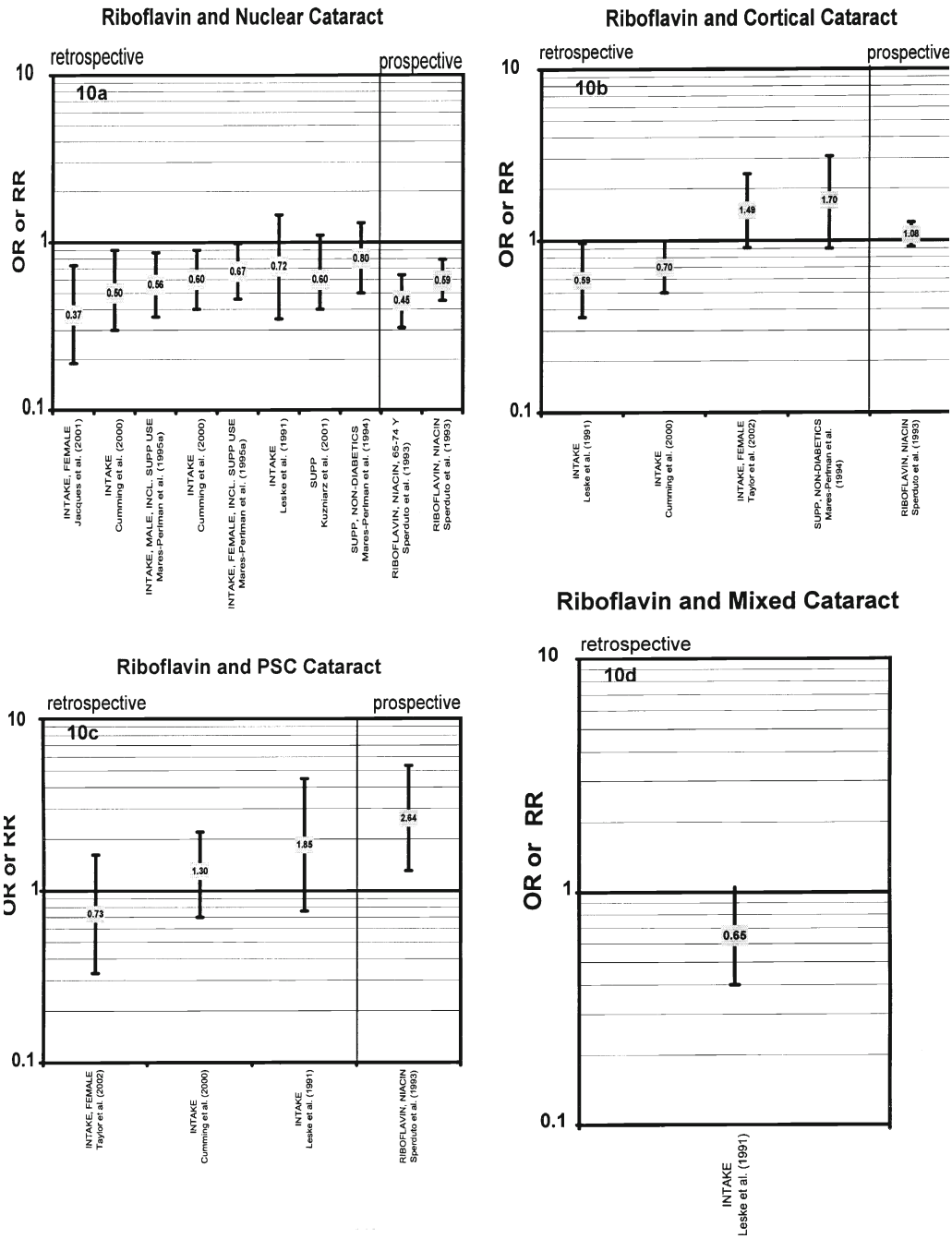

Fig. 10. Riboflavin and cataract risk ratio for high vs low intake (with or without supplements), blood levels, or supplement use. Data for retrospective and prospective studies are presented independently. Log scale.

from a study by Mares-Perlman et al. *(92)* suggested a 70% increased rate of cataract (RR = 1.7, 95% CI 0.9–3.1; *p*-trend = 0.05) in nondiabetics with the highest vs lowest riboflavin supplements.

3.4.5.3. Posterior Subcapsular. Four studies examined relationships between riboflavin and risk for PSC cataract. Three studies produced no effect *(82,89,99)*. However, in an intervention study of 3249 subjects, Sperduto et al. *(120)* found a 2.6-fold increase in the rate of cataract (RR = 2.64, 95% CI 1.31–5.35) between subjects who used the niacin and riboflavin supplement and those who did not.

3.4.5.4. Mixed. The only study that examined relationships between riboflavin intake and risk for mixed cataract did not find statistically significant data *(89)*.

3.5. Observational Studies Regarding Multivitamins and Antioxidant Index

In addition to multivitamin supplement use, this section includes studies using "antioxidant indices" that approximate combined effects of the multiple antioxidants contained in food intake on risk for cataract. However, single nutrients appear to have strong influences on the indices, and we now question the usefulness of the indices.

3.5.1. Nuclear

Ten studies examined relationships between multivitamins or antioxidant index and risk for nuclear cataract. Six studies, including the Linxian intervention, found statistically significant data supporting the hypothesis that increased multivitamin supplement use or antioxidant index intake is related to diminished risk for nuclear cataract *(88,89,92,111,115,120)*. The remaining four studies showed no effect *(87,91,93,101)*.

Kuzniarz et al. *(88)* found a 90% reduced rate of nuclear cataract (RR = 0.1, 95% CI 0–1.0; *p*-trend = 0.02) between subjects who did and did not use multivitamin supplements. Leske et al. *(89)* found a 45% reduced rate of cataract (RR = 0.55, 95% CI 0.33–0.92) in users of multivitamin supplements and, in her prospective study, found a 31% reduced rate of cataract (RR = 0.69, 95% CI 0.48–0.99) in users of multivitamin supplements *(111)*.

Mares-Perlman et al. *(92)* found a 40% reduced rate of cataract (RR = 0.6, 95% CI 0.5–0.8) in nondiabetics with the highest and lowest multivitamin supplements and a 40% reduced rate of cataract (RR = 0.6, 95% CI 0.4–0.9) between subjects who did and did not use multivitamins for more than 10 yr *(115)*. Sperduto et al. *(120)* found a 43% reduced rate of cataract (RR = 0.57, 95% CI 0.36–0.9) in 65- to 74-yr-old Chinese people who used multivitamins for 6 yr.

3.5.2. Cortical

Nine studies examined relationships between multivitamins or antioxidant index and risk for cortical cataract. Four studies found statistically significant data supporting the hypothesis that increased multivitamin supplement use or antioxidant index intake or blood levels is related to diminished risk for cortical cataract *(85,89,114,115)*. One study found statistically significant data indicating that increased multivitamins supplement use is related to increased risk for cortical cataract *(92)*. The remaining four studies, including the Linxian intervention, showed no effect *(91,99,101,120)*.

In a retrospective study of 112 subjects, Jacques and Chylack *(85)* found a 84% reduced rate of cortical cataract (OR = 0.16) between subjects with the highest and lowest antioxidant index. Leske et al. *(89)* found a 58% reduced rate of cortical cataract

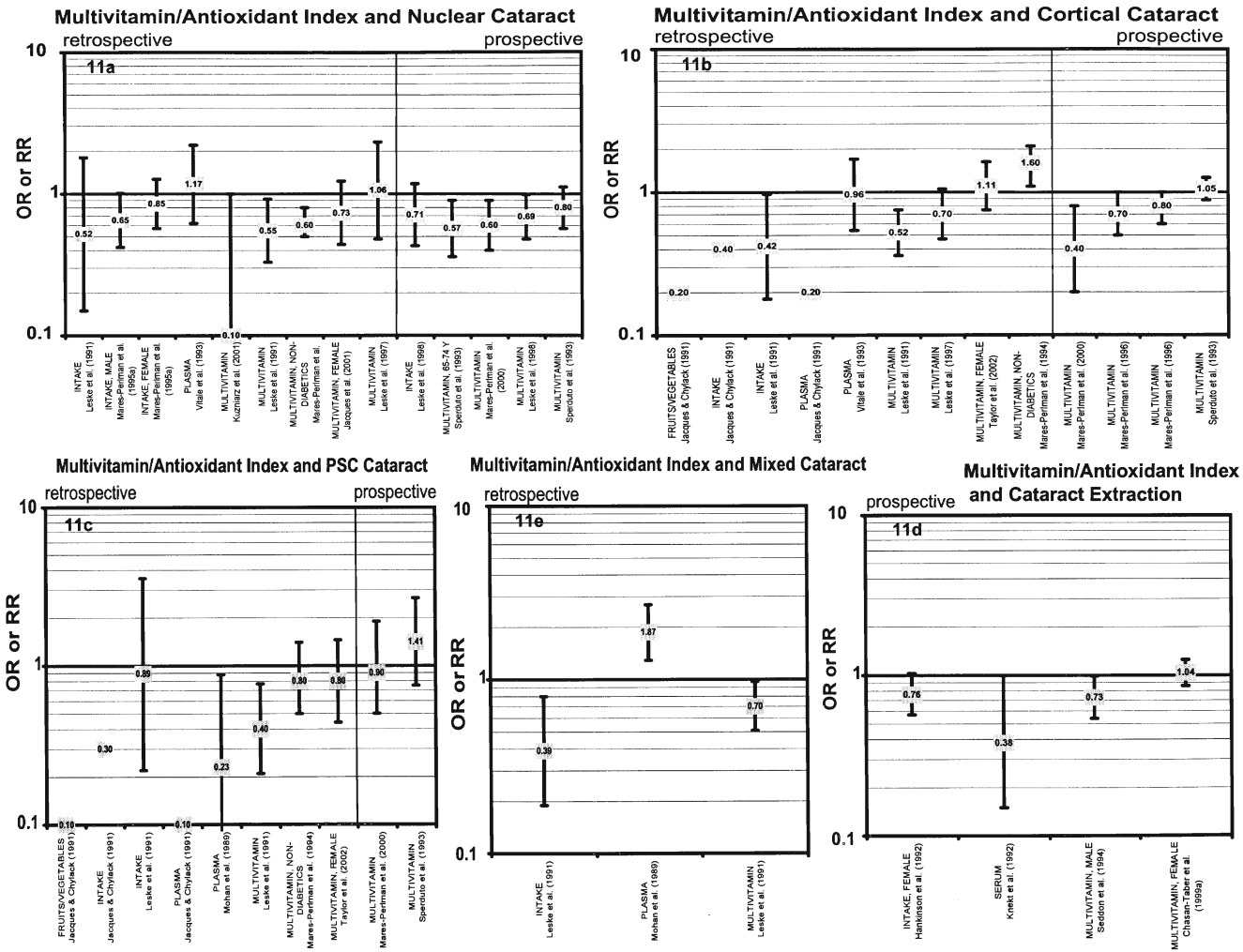

Fig. 11. Multivitamins/antioxidant index and cataract risk ratio for high vs low intake (with or without supplements), blood levels, or supplement use. Data for retrospective and prospective studies are presented independently. Log scale.

(RR = 0.42, 95% CI 0.18–0.97) between subjects who did and did not use the highest and lowest antioxidant intake index and found a 48% reduced rate of cataract (RR = 0.52, 95% CI 0.36–0.75) among subjects who did and did not use multivitamin supplements.In a retrospective study of 1862 subjects, Mares-Perlman et al. *(92)* found a 60% increased rate of cortical cataract (RR = 1.6, 95% CI 1.1–2.1) in nondiabetics with the highest vs lowest multivitamin supplements.

In contrast, in a prospective study of 3220 subjects, Mares-Perlman et al. *(114)* found a 30% reduced rate of cataract incidence (RR = 0.7, 95% CI 0.5–1.0) and a 20% reduced rate of cataract progress (RR = 0.8, 95% CI 0.6—1.0) between users and nonusers of multivitamin supplements. In a more recent prospective study of 2434 subjects, Mares-Perlman et al. *(115)* found a 60% reduced rate of cataract (RR = 0.4, 95% CI 0.2–0.8; *p*-trend = 0.002) in subjects who took multivitamin supplements or supplements of vitamin C or E for more than 10 yr.

3.5.3. Posterior Subcapsular

Seven studies examined relationships between multivitamins or antioxidant index and risk for posterior subcapsular cataract. Three studies found statistically significant data supporting the hypothesis that increased multivitamin supplement use or antioxidant index intake or blood levels is related to diminished risk for PSC cataract *(85,89,96)*. The remaining four studies, including the Linxian intervention, showed no effect *(92,99,115,120)*.

Jacques and Chylack *(85)* found that adjusted prevalence for PSC cataract was decreased by 80% (RR = 0.16, 95% CI 0.04–0.82) for persons with mgh antioxidant index scores, based on combined intake of vitamins C and E and carotenoids. Leske et al. *(89)* found a 60% reduced rate of cataract (RR = 0.4, 95% CI 0.21–0.77) among users of multivitamin supplements, and Mohan et al. *(96)* found a 77% reduced rate of PSC cataract (RR = 0.23, 95% CI 0.06–0.88) between subjects with the highest and lowest antioxidant indices in a retrospective study of 1990 subjects.

3.5.4. Mixed

Two studies examined relationships between multivitamins or antioxidant index and risk for mixed cataract. Leske et al. *(89)* found a 61% reduced rate of cataract (RR = 0.39, 95% CI 0.19–0.8) between subjects with the highest and lowest antioxidant intake indices and found a 30% reduced rate of cataract (RR = 0.7, 95% CI 0.51–0.97) in users of multivitamin supplements. In a retrospective study of 1990 subjects, Mohan et al. *(96)* found an 87% increased rate of cataract (RR = 1.87, 95% CI 1.29–2.69) between subjects with the highest and lowest antioxidant plasma indices.

3.5.5. Extraction

Four studies examined relationships between multivitamins or antioxidant index and risk for cataract extraction. Knekt et al. *(110)* found a 62% reduced rate of cataract (RR = 0.38, 95% CI 0.15–1.0) between subjects with the highest and lowest levels of vitamin E and β-carotene in the multivitamin serum. In a prospective study of 17,744 subjects, Seddon et al. *(119)* found a 27% reduced rate of cataract (RR = 0.73, 95% CI 0.54–0.99) between physicians who did and did not use multivitamins—but not vitamin C or E supplements alone. The remaining two studies produced no effect *(104,108)*.

4. CONSIDERATIONS FOR FUTURE RESEARCH

Because intake or plasma measures are highly variable and the effects of diet are likely to be cumulative, studies should be performed on populations for which long-term dietary records or multiple measures of plasma levels of nutrients are available. If a single measure of status must be chosen, intake studies seem to be preferable to the use of plasma measures. Identifying the best means to quantify nutrient intake also would be beneficial.

Comparison of information from the NVP substudy of the Nurse's Health Study indicates that the greatest opportunity to observe benefits from antioxidants is when cataracts are at an early stage. This is also supported by comparison of results from the American vs English arms of the REACT trial. Additionally, our results and those of several other investigators, including the ARM of the AREDS trial, clearly indicate that modified and consistent intake over prolonged periods is essential to observe benefits from nutrients.

Further definition and refinement of techniques to quantify progress of cataract (and AMD) would be helpful, and greater ability to compare among studies would be invaluable regarding analysis of data from multiple studies.

5. CONCLUSIONS AND RECOMMENDATIONS

Why study relationships between risk for cataract and nutrition when there are already economic, safe surgical procedures to replace and remediate the opacified lens in the United States? There are several important reasons, including: (a) it is likely that the same nutritional practices that are associated with prolonged lens function will also be associated with delayed age-related compromises to other organs and, perhaps, aging *per se*; (b) there are not sufficient surgical resources to provide economic, safe surgeries worldwide; and (c) there will be considerable financial savings and improvements in quality of life if health, rather than old age, is extended—particularly given the rapidly growing elderly segment of our population. For instance, data from the AMD arm of the AREDS intervention trial predict that if persons with moderate AMD take the AREDS supplement, 350,000 cases of blindness may be avoided over 5 yr (Table 2) *(102)*.

Oxidative stress clearly is involved in the etiology of cataract. This involves both damage to the structural proteins of the lens as well as compromises to the proteolytic machinery that might otherwise remove the damaged proteins that aggregate and form cataractous precipitates. Given the correlation between oxidative damage and this age-related debility, it is not surprising that more than 25 studies have attempted to relate antioxidant intake to risk for cataract. Although data from the observational studies generally is consistent in finding a benefit to be gained from maintaining higher levels of antioxidants either in diet or plasma, the data from the intervention trials are far less encouraging regarding limiting risk for cataract prevalence or cataract progress. Without more information, it is difficult to parse these results. Are the observational studies flawed? Are the intervention trials less than what might be expected from the ideal exploration? Were any of them of long enough duration to provide the data sought?

It seems clear that good nutrition (based on diets that are rich in fruits and vegetables), coupled with healthy lifestyles (which preclude known risk factors for eye disease such as smoking, excessive weight, and poverty) must be started early in life to obtain the benefit of prolonged sight later in life. Because the same dietary practice also optimizes many aspects of health, including immune function, there should be good incentive to strive for healthier practices.

If good nutrition and sufficient resources for lifestyles that are associated with diminished risk for cataract are not available (i.e., among the impoverished), some supplementation might be considered. Started early in life, dietary intake of about 250, 90, and 3 mg/d of vitamins C and E and lutein, respectively, should provide sufficient reserves to provide the health benefit. These levels are considerably higher than the current RDAs. The lutein recommendation is based on our observational data *(87)*. To date, lutein has not been tested in any of the interventions published. Because the bioavailability of ascorbate may decrease with age, slightly higher intakes might be required in the elderly. Defining useful levels of the other nutrients requires further studies.

Although women appear to be more prone to eye disease than men *(95,123)*, there is presently no reason to think that nutrient intake or blood levels of nutrients would affect the genders differently.

Levels of β-carotene in the lens are below detection limits. This calls into question prior observations that this nutrient is associated with lens health. Together with data from the ATBC Study, which indicated higher levels of cancer in smokers who took supplements of β-carotene, it would appear that there is no reason to use supplements of this carotenoid and that the original data that associated diminished risk for cataract with elevated intake of this nutrient should be re-examined. However, if relationships are found between these carotenoids and lens health, then this would imply a mechanism of action that includes activities other than direct antioxidant function. This is a useful concept to retain as we search for other agents to prolong lens function. Therefore, we have started testing associations between dietary pattern and eye health.

In conclusion, the data from the diverse epidemiological and biochemical studies suggest that in widely disparate tissues, there are many common insults and mechanistic compromises associated with aging and that proper nutrition early in life may address some of these compromises and provide extended youthful function later in life. Indeed, proper nutrition, possibly including use of antioxidant vitamin supplements for the nutritionally impoverished, along with healthy lifestyles (*see* earlier) may provide the least costly and most practical means to delay cataract.

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20 Antioxidant Nutrients and Prevention of Oxidant-Mediated Diseases

Ronald Anderson

KEY POINTS

- Chronic exposure to excessive levels of atmospheric pollution in the workplace, environment, and home (especially from cigarette smoke) is accompanied by increased oxidative stress.
- Inhalation of cigarette smoke has a profoundly irritant effect on phagocytic cells, causing an increase in both the number and oxidant-generating activities of these cells.
- Smoking-induced oxidative stress is associated with increased turnover of the antioxidant nutrients vitamin C, β-carotene, and vitamin E in the circulation and lungs.
- Relatively moderate increases in the numbers of circulating phagocytes has consistently been shown to be an independent predictor of decline in pulmonary function, development of several cardiovascular conditions (including myocardial infarction, sudden cardiac death, all coronary heart disease (CHD) combined, stroke, and essential hypertension), lung cancer incidence and mortality, possibly cancer at all sites, and death from all causes. Adequate intake of vitamin C, vitamin E, and β-carotene is necessary to counteract the development of oxidant-mediated tissue damage, organ dysfunction, and disease.

1. INTRODUCTION

Certain high-risk occupations, adverse socioeconomic and sociocultural circumstances, and unhealthy, avoidable aspects of lifestyle may—individually or collectively—result in heightened levels of oxidative stress, predisposing the individual to future development of oxidant-mediated organ dysfunction and disease *(1,2)*. Outdated and/or poorly regulated industrial practices; unacceptably high levels of vehicle exhaust emissions; overpopulation associated with overcrowded, poorly ventilated, nonelectrified dwellings; and poor dispersal of atmospheric pollutants caused by unfavorable climate conditions and/or topography are problems commonly encountered in, but not limited to, many developing countries *(3,4)*. In these circumstances, chronic exposure to excessive levels

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of atmospheric pollution in the workplace, environment, and home is accompanied by increased oxidative stress. Unhealthy lifestyles such as poor dietary habits (especially low intake of fresh fruits and vegetables *[5]*), cigarette smoking *(2)*, and, in some cases, excessive exposure to ultraviolet radiation *(6)* also accelerate the onset of those degenerative diseases (cataracts, cardiovascular diseases [CVDs], cancer, pulmonary dysfunction, and emphysema) that have a suspected oxidant-mediated etiology *(1,2)*.

Cigarette smoking is the most common and eminently avoidable cause of lifestyle-related oxidative stress associated with accelerated onset of degenerative disease and premature death. Moreover, the toxicology of cigarette smoke inhalation is probably broadly similar to that of many other inhaled, pro-oxidative irritants. For these reasons, this chapter focuses primarily on cigarette smoking to review the relationships that exist between atmospheric pollution, nutrition, and oxidant-mediated diseases.

2. CIGARETTE SMOKING

The magnitude of the ongoing, and apparently increasing, threat posed to public health by cigarette smoking is emphasized by the following comment, which remains valid 10 yr after being recorded:

> *Smoking represents a great failure in public health; more than 40 yr after the hazards were first established, cigarettes are still responsible for 30% of deaths in middle age in Britain and the United States, and worldwide sales are increasing (7).*

This statement is based on data derived from an epidemiological study designed to investigate mortality in relation to smoking over a 40-yr period in British male doctors *(8)*. Alarmingly, the results of this study demonstrated that the hazards of long-term use of tobacco were substantially underestimated in previous studies conducted over shorter periods; a revised estimate shows that about half of all regular cigarette smokers will eventually be killed by their habit *(8)*. The average decrease in life expectancy of smokers relative to nonsmokers is 8 yr *(8)*. If current smoking trends persist, it is predicted that smoking will be one of the largest causes of premature death worldwide *(7)*.

2.1. Origins of Oxidants During Smoke Exposure

There are two major sources of oxidative stress in smoke-exposed individuals. First, the gas and tar phases of cigarette smoke contain extremely high levels of organic radicals; the respective, approximate concentrations are 10^{15}/puff and 10^{17}/g. Second, inhalation of cigarette smoke has a profoundly irritant effect on the phagocytic cells of the immune system, causing an increase in both the number and oxidant-generating activities of these cells *(9–11),* and the high iron content of cigarette smoke may favor generation of the highly toxic hydroxyl radical by these cells in the airways of smokers *(12)*. Consequently, chronic exposure of the smoker to high levels of reactive oxidants is accompanied by an increased risk of oxidant-mediated diseases. These events are summarized in Fig. 1.

2.2. Proinflammatory Effects of Cigarette Smoking

Cigarette smoking causes an acute localized inflammatory reaction characterized by the accumulation of phagocytes (neutrophils and macrophages) in the membranous bronchioles and alveoli of the lungs, leading to destruction of the peribronchiolar alveolar attachments and pulmonary dysfunction *(13,14)*. However, these inflammatory

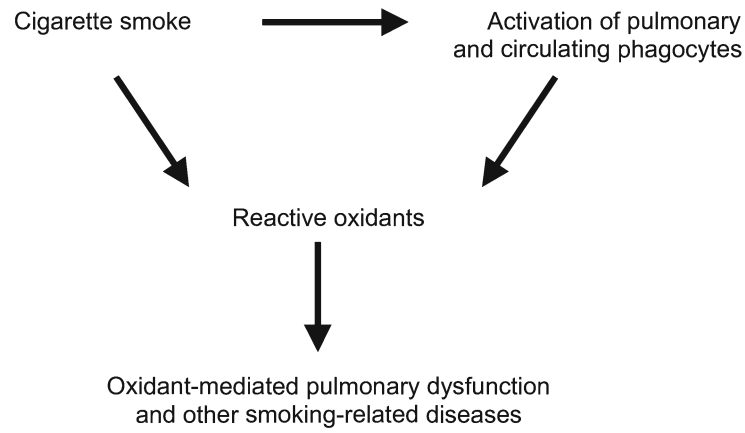

Fig. 1. Events leading to pulmonary dysfunction in smokers.

events are not confined to the lung, and systemic, proinflammatory effects of cigarette smoking have also been demonstrated in numerous studies. Compared to nonsmokers, cigarette smokers have significantly increased circulating leukocyte counts, which are inversely correlated with the degree of airflow limitation *(9,15)*. Additionally, the decline in forced expiratory flow in 1 s (FEV_1), an important measure of pulmonary function, is inversely correlated with both the initial peripheral leukocyte count *(16)* and the change in leukocyte count over time, independently of the smoking habit *(17)*. The normal tempo of neutrophil production in the bone marrow is about 0.5 to 1.5×10^9/kg of body weight daily. These short-lived phagocytes are released into the circulation and account for up to 70% of the circulating leukocyte population. The normal range for circulating leukocyte counts in apparently healthy adult humans is 4 to 11×10^6 mL of blood, which is an underestimate because approx 50% of the circulating neutrophil pool is adherent to vascular endothelium. On average, cigarette smoking increases the numbers of circulating leukocytes (mainly neutrophils and monocytes) by 20 to 30%; however, in some cases, the increase may exceed 100%. Cigarette smoking not only increases the numbers of circulating and pulmonary phagocytes but also enhances the pro-oxidative and adhesive properties of these cells. Phagocytes from cigarette smokers are sensitized for increased production of reactive oxidants *(11,12,18)*, which is attributable, at least in part, to increased content of the pro-oxidative enzyme myeloperoxidase (MPO) *(20)* and is closely correlated with the degree of pulmonary dysfunction *(19)*. Cigarette smoke-mediated activation of neutrophils in vivo causes delayed transit of these cells through the pulmonary microcirculation *(21,22)*, which is probably a result of oxidant-mediated adhesion of these cells to vascular endothelium *(23,24)*, altered expression of cellular adhesion molecules *(25)*, intravascular aggregation of neutrophils and platelets *(23,26)*, and increased release of MPO and elastase *(22,27)*.

3. HARMFUL ACTIVITIES OF OXIDANTS

Phagocyte-derived reactive oxidants, as well as those present in cigarette smoke, have a range of harmful activities. These oxidants have been reported to be cytotoxic for a wide variety of eukaryotic cells *(28,29)* and are also immunosuppressive, carcinogenic, proproteolytic, proadhesive, and proatherogenic. The biochemical mechanisms

Table 1
Mechanisms Involved in the Etiology of Oxidant-Mediated Disorders

Disease	*Pro-oxidative mechanism*
Cancer	Oxidative damage to DNA Inactivation of DNA repair enzymes
Pulmonary emphysema and tissue damage in autoimmune diseases such as rheumatoid arthritis	Potentiation of the activity of phagocyte-derived proteases
Cardiovascular disease	Oxidative modification of low-density lipoprotein Adhesion of phagocytes to vascular endothelium
Acquired immunosuppression	Oxidative inactivation of the protective activities of B and T lymphocytes, as well as natural killer cells

involved in the etiology of oxidant-mediated disorders are summarized in Table 1. Whereas the relative contributions of oxidants derived directly from cigarette smoke and those from smoke-activated phagocytes to the oxidative events involved in the pathogenesis of smoking-related diseases are unknown; the combined assault from these two sources is unremitting and difficult to counteract.

3.1. Oxidants as Carcinogens

Given the complexity and range of reactive oxidants that it contains, it is hardly surprising that several pro-oxidative mechanisms have been proposed to account for the observed direct DNA-damaging effects of cigarette smoke *(2,29,30)*. It has been proposed that polyhydroxy aromatic compounds present in tar bind to the genetic material, leading to generation of hydrogen peroxide (H_2O_2), which in turn causes DNA strand breaks by a hydroxyl radical ($^{\bullet}OH$)-dependent mechanism *(30)*.

The association between chronic inflammation, phagocyte-derived oxidants, and development of epithelial cancers is well recognized *(31)*. Activated phagocytes have been identified as potential carcinogens because they oxidatively damage DNA and promote malignant transformation in bystander cells in tissue culture *(22)*. Hydrogen peroxide is the oxidant primarily responsible for phagocyte-mediated DNA damage to neighboring cells. This permeant oxidant interacts with intracellular transition metals to generate $^{\bullet}OH$ in close proximity to DNA, leading to oxidative damage to adenine, guanine, thymine, and cytosine as well as DNA strand breaks *(31)*. Although DNA damage in living cells is subject to cellular repair, it may occasionally escape repair, or repair may be incorrect. In such cases, unrepaired or misrepaired DNA could have deleterious consequences, leading to gene modifications that may ultimately promote cellular transformation *(32)*. Ominously, it also has been reported that the permeant, phagocyte-derived reactive oxidant hypochlorous acid (HOCl) oxidatively inactivates the DNA repair enzyme poly(ADP ribose) polymerase in bystander cells exposed to activated neutrophils *(33)*, indicating that phagocyte-derived oxidants not only damage DNA but also compromise DNA repair mechanisms.

3.2. Proteolytic Activities of Oxidants

Reactive oxidants present in cigarette smoke as well as those released by activated phagocytes have been reported to potentiate the activity of neutrophil-derived proteolytic enzymes. Neutrophil granules contain a large family of more than 20 enzymes; however, 4 proteolytic enzymes (the neutral serine proteases elastase and proteinase 3 and the two metalloproteinases collagenase and gelatinase) seem to have the greatest potential to act as mediators of tissue injury *(34)*. These proteases are released extracellularly by activated neutrophils *(27)*, and each cleaves key components of the extracellular matrix, which is composed of a complex mix of collagens, elastin, proteoglycans, and glycoproteins and lies under epithelia and surrounds connective tissue cells *(34)*. Extracellular release of proteases and generation of reactive oxidants by activated neutrophils are concomitant and interrelated events. Reactive oxidants, especially phagocyte-derived HOCl *(34)*, as well as poorly defined oxidants present in cigarette smoke *(2)* dramatically potentiate the proteolytic activity of these neutrophil proteases by direct and indirect mechanisms. In the case of elastase and proteinase 3, HOCl and cigarette smoke promote the oxidative inactivation of α-1-protease inhibitor (API), the major plasma and tissue inhibitor of these enzymes. On the other hand, collagenase and gelatinase are secreted in a latent form by neutrophils and undergo oxidative activation on exposure to HOCl *(34,35)*. When oxidatively activated, collagenase and gelatinase potentiate the activity of elastase and proteinase 3 by cleaving API within its active site loop, causing irreversible inactivation of this protease inhibitor *(34,35)*. The consequence is uncontrolled elestolysis in the lungs of cigarette smokers, leading to pulmonary dysfunction and emphysema.

3.3. Proatherogenic Properties of Oxidants

Reactive oxidants are believed to be intimately involved in the pathogenesis of atherosclerosis by promoting oxidative modification of low-density lipoprotein (LDL), which then accumulates in the arterial intima and appears to be the major contributor to the formation of the atherosclerotic lesion *(36)*. Oxidized LDL is selectively chemotactic for monocytes/macrophages and, unlike native LDL, is taken up by these cells, resulting in the formation of cytokine-producing foam cells *(36)*. Interestingly, oxidized LDL has been described to be immunogenic, initiating the formation of autoantibodies against neoepitopes on the oxidatively modified molecule *(36,37)*. The nature of the involvement (i.e., primary or secondary) of these autoantibodies in the etiology and progression of atherosclerosis remains to be established.

There are several possible mechanisms by which inhalation of cigarette smoke may promote oxidative modification of LDL, including direct oxidation of this molecule by smoke-derived oxidants. However, because plasma contains high concentrations of antioxidants, it seems improbable that meaningful oxidation of LDL would occur in the circulation. Alternatively, cigarette smoking may predispose to atherosclerosis as a secondary consequence of accelerated consumption of circulating nutritional antioxidants, such as ascorbate and β-carotene, rendering LDL vulnerable to phagocyte- and endothelial cell-derived reactive oxidants as it passes through the arterial wall *(36,38)*.

3.4. Proadhesive Activity of Oxidants

Exposure of vascular endothelium to superoxide (O_2^-) or H_2O_2 in vitro induces prolonged expression of the adhesion molecules P- and E-selectins on endothelial cells,

with resultant adherence of neutrophils *(23,24)*. Oxidant-mediated activation of neutrophil adhesion to vascular endothelium probably is intimately involved in the pathogenesis of postichemic vascular injury. In this setting, however, the proadhesive oxidants originate primarily from the endothelial cells. Ischemia and hypoxia promote the proteolytic conversion of xanthine dehydrogenase to the superoxide-generating enzyme xanthine oxidase in endothelial cells. Subsequent activation of this enzyme during reperfusion/reoxygenation leads to generation of O_2^- and H_2O_2:

These oxidants (O_2^- and H_2O_2) not only cause direct vascular injury but also upregulate expression of P- and E-selectins on vascular endothelium, leading to adherence and activation of neutrophils, which contribute to vascular damage by pro-oxidative mechanisms. These events may explain the involvement of neutrophils in the exacerbation of myocardial damage that accompanies restoration of circulation and reoxygenation during the postinfarct period.

An additional mechanism of oxidant-mediated proadhesive activity was described in smoke-exposed rodents. Using a dorsal skinfold chamber combined with intravital fluorescence microscopy to study the microcirculation in fine-striated skin muscle, it was demonstrated that exposure of hamsters to cigarette smoke resulted in rapid adhesion of leukocytes to the endothelium of postcapillary venules and arterioles as well as the formation of intravascular aggregates of leukocytes and platelets *(23)*. Although the exact mechanisms of these smoke-induced adhesive interactions between circulating leukocytes and vascular endothelium were not established, it was suggested that reactive oxidants present in cigarette smoke may have upregulated expression of P-selectin on endothelial cells *(23)*. These proadhesive events are probably mechanistically involved in the etiology of smoking-related pulmonary disease and CVDs.

3.5. Immunosuppressive Properties of Oxidants

Reactive oxidants are also potent antiproliferative agents. Permeant oxidants, such as H_2O_2 and HOCl, inhibit the proliferative activity and functions of B and T lymphocytes and natural killer (NK) cells in vitro, probably by interfering with the activity of several enzymes involved in cellular energy metabolism *(39,40)*. Smoking-related pro-oxidative events may compromise pulmonary defense mechanisms, because smokers have decreased antibody responses to inhaled microbial antigens as well as abnormal NK function *(41,42)*. However, the precise mechanisms and relative contributions of smoke- and phagocyte-derived oxidants to smoking-related immunosuppression remain to be established.

4. ANTIOXIDANT NUTRIENTS AND SMOKING-RELATED DISEASES

It is abundantly clear that radical and nonradical reactive oxidants are intimately involved in the etiology of smoking-related pulmonary dysfunction, bronchial carcinoma, and cardiovascular disorders. The rate at which these diseases develop in individual smokers probably involves a dynamic interplay between oxidants and other toxins present in cigarette smoke, the numbers and pro-oxidative activities of circulating and resident pulmonary phagocytes, and the efficiency of the smoker's antioxidant defenses *(43–45)*.

Moreover, oral administration of vitamin C to active smokers as well as to individuals exposed to environmental tobacco smoke resulted in a decline in the concentrations of

circulating and intracellular biomarkers of oxidative stress, as well as attenuation of activation of genes encoding antioxidative enzymes such as catalase, glutathione peroxidase, and superoxide dismutase *(46,47)*. These observations suggest that inadequate intake of vitamin C, and possibly vitamin E and β-carotene, may be determinants of susceptibility to development of smoking-related diseases *(43–45)*.

4.1. Vitamin C

The negative impact of cigarette smoking on plasma vitamin C levels is well-recognized *(43)* and is caused, at least in part, by increased turnover of the vitamin, which explains the observed dose–response relationship between the number of cigarettes smoked per day and the decline in plasma vitamin C *(48,49)*. Neutralization of oxidants present in cigarette smoke *(43)* as well as those released by smoke-activated phagocytes *(50)* are the probable mechanisms of accelerated consumption of the vitamin. Because vitamin C has been demonstrated to protect API against oxidative inactivation by both cigarette smoke *(51)* and activated phagocytes *(52)* in vitro and to prevent activation of latent metalloproteinases *(53)* and cigarette smoke-mediated adhesion of leukocytes to vascular endothelium in hamsters *(23)*, this vitamin probably is critically involved in protecting the lungs against oxidant-inflicted damage and dysfunction. Indeed, data from an epidemiological study highlighted a clear and significant positive correlation between dietary intake of vitamin C and pulmonary competence *(54)*. Interestingly and importantly, the association between vitamin C and pulmonary function persisted after adjustment for cigarette smoking, indicating that this protective activity of the vitamin is not limited to smoke-mediated oxidative stress *(54)*. This contention is supported by observations that deficiencies of vitamins C and E are associated with wheezing symptoms in both smokers and nonsmokers *(55)*.

4.2. Vitamin E and β-Carotene

Vitamin E has also been reported to regulate both the production and reactivity of reactive oxygen species by activated neutrophils in vitro and ex vivo *(56–58)*, whereas β-carotene is a scavenger of phagocyte-derived singlet oxygen *(59)* and HOCl *(57)*. Although neither of these antioxidants appears to be an efficient scavenger of smoke-derived oxidants *(38)*, dietary intake of vitamin E and β-carotene, similarly to vitamin C, may be a determinant of pulmonary competence in cigarette smokers by protecting the lungs against the destructive effects of smoke-activated phagocytes. This contention was supported by data from a study that described significant positive correlations between plasma levels of β-carotene and several spirometric parameters in asymptomatic, male cigarette smokers but not in nonsmokers *(60)*. Distinct relationships between plasma levels of vitamin E and pulmonary function were also reported in both smokers and nonsmokers *(61)*. In nonsmoking males, plasma vitamin E was positively and significantly correlated with pulmonary competence, whereas, somewhat surprisingly, inverse correlations were observed between these parameters in a matched group of cigarette smokers *(61)*. It was proposed that in the physiological setting (typified by nonsmokers), maintenance of vitamin E homeostasis is probably adequate to protect the lungs against routine, environmental, and endogenous oxidative stress, accounting for the positive relationship with pulmonary function. However, sustained and excessive oxidative stress may necessitate mobilization of tissue stores of the vitamin and diversion to the lungs, which would explain the apparent inverse association between plasma

vitamin E and pulmonary competence in smokers *(61)*. This type of mobilization of vitamin E to the lungs was described in rats experimentally exposed to cigarette smoke *(62)* and ozone *(63)*.

4.3. Antioxidant Nutrients and CVD

An association between adequate dietary intake of β-carotene and the antioxidant vitamins (particularly vitamin E) and reduced incidence of and mortality from CVD has been demonstrated in a number of large epidemiological studies *(64–67)*. Although smoking has been identified as a major risk for development of CVD, these associations persisted after adjustment for smoking status, demonstrating the predisposing role of additional factors, especially low dietary intake of these antioxidant nutrients. Moreover, physiological levels of vitamin C were reported to inhibit the oxidative modification of LDL in vitro *(68)*, whereas short-term oral administration of relatively high doses of this vitamin or of vitamin E prevented *(69)* or retarded *(36)* peroxidative alteration of LDL ex vivo. Identification of autoantibodies to oxidized LDL in individuals with atherosclerosis and the possible involvement of these in the etiology of this condition *(70)* suggests that antioxidant nutrient status may determine the susceptibility of LDL to oxidative, autoantigenic modification. To support this finding, we detected increased levels of circulating autoantibodies to both oxidized LDL and cardiolipin in asymptomatic cigarette smokers older than age 30 yr. The levels of these autoantibodies were significantly and inversely correlated with plasma levels of vitamin C, indicating that intake of this antioxidant nutrient may be a determinant of development of atherosclerosis in smokers *(71)*.

There is mounting and compelling evidence that antioxidants, particularly vitamin C, at relatively high intakes can both prevent and restore the endothelial dysfunction that leads to the development of CVDs such as hypertension, diabetes, atherosclerosis, idiopathic dilated cardiomyopathy, and congestive heart failure *(72)*. Although they are not exactly clear, there are several—albeit probably related—mechanisms by which vitamin C improves the defective endothelium-dependent vasodilatation that underlies these cardiovascular disorders. These include antagonism of endothelial cell apoptosis *(73)*; protection of nitric oxide (NO), an important regulator of vasomotor tone, against oxidative inactivation *(74)*; potentiation of NO synthesis *(75,76)*; antagonism of homocysteine *(77)*; inhibition of the production of proatherogenic phospholipids *(78,79)*; and prevention of the pro-oxidative and proapoptotic effects of hyperglycemia *(80–82)*. These protective effects of vitamin C on endothelial integrity and function are likely to involve, at least in part, vitamin-mediated neutralization of phagocyte-derived reactive oxidants *(69,79,80)*.

4.4. Antioxidant Nutrients and Cancer

Decreased dietary intake and/or accelerated metabolism of the antioxidant vitamins and β-carotene during sustained oxidative stress probably is a common cause of cancer *(83)*. Cigarette smoking is the primary cause of lung cancer and is also associated with the development of cancer at several other sites (mouth, larynx, esophagus, bladder, pancreas) *(7)*. Significant inverse correlations between the dietary intake and/or plasma levels of the antioxidant nutrients and development of cancer at various sites have been reported in numerous epidemiological studies. The consistency of these reports in conjunction with corroborative laboratory studies has prompted requests for

the implementation of nutrient-based preventive public health strategies *(5,83)*. However, these laboratory and epidemiological studies have not been supported by data from some of the completed antioxidant nutrient intervention studies *(84,85)*. Although aspects of the design of these intervention studies may have inadvertently obscured the possible preventive effects of vitamin C, vitamin E, and β-carotene, it is clear that definitive answers about the anticancer properties of these agents await the outcome of large-scale ongoing studies. Moreover, detection of beneficial effects of antioxidants may require intervention at a relatively young age with monitoring over several decades, as opposed to intervention in middle-age spanning only several years *(86,87)*.

5. PREDICTORS OF OXIDANT-MEDIATED DISEASE

Although cigarette smoking has been used in this chapter as the prototype cause of lifestyle-related oxidative stress, the mechanisms of smoking-associated oxidant-mediated tissue damage and disease probably are broadly operative in other settings, including environmental and occupational exposure to excessive levels of atmospheric pollution. Even in these settings, however, active *(88)* and passive *(89)* exposure to cigarette smoke often may be the primary offender.

Interestingly, the inverse relationships between dietary intake and/or plasma levels of the antioxidant nutrients vitamin C, vitamin E, and β-carotene and future development of degenerative disease and cancer described in major epidemiological studies remain after adjustment for smoking history. Therefore, there must be a common mechanism that is operative in both smokers and nonsmokers but that is clearly exacerbated by cigarette smoking, which determines susceptibility to oxidant-mediated disease. This mechanism may involve a dynamic interplay between dietary intake of antioxidant nutrients and the numbers and reactivities of the abundant, highly aggressive, oxidant-generating phagocytic cells of the immune system. Although critically involved in host defense against microbial pathogens, the sheer numbers of these cells and the undiscerning nature of their arsenal of toxic antimicrobial oxidants constitutes an unrelenting threat to other host cells and tissues. Coexistence between the host and the phagocytic cells of his of her immune system is probably fragile, and minor imbalances may compromise the containment (damage limitation) functions of vitamins C and E and β-carotene, creating the potential hazard of phagocyte-inflicted oxidative damage.

Such imbalances may be caused by several common, often avoidable, aspects of lifestyle that disrupt oxidant/antioxidant homeostasis. Poor dietary habits may impair antioxidant defenses through decreased intake of the antioxidant vitamins and β-carotene, whereas cigarette smoking, occupational and environmental atmospheric pollution, and excessive exposure to ultraviolet radiation *(6)* may cause a futile, potentially harmful increase in the numbers and pro-oxidative activities of resident and circulating phagocytes.

5.1. Phagocytes, Antioxidants, and Degenerative Disorders

The health threat posed by sustained, relatively moderate increases in the numbers of circulating phagocytes is emphasized by evidence from numerous epidemiological studies that have consistently shown that the circulating leukocyte count, and, particularly, the neutrophil count, measured well before the onset of manifest clinical disease is an independent predictor of decline in pulmonary function *(17)*, development of several cardiovascular conditions (including myocardial infarction, sudden cardiac death,

all CHD combined, stroke, and essential hypertension *[90–92]*), lung cancer incidence and mortality, possible development of cancer at all sites *(93,94)*, and death from all causes *(95)*. For each decrease in the circulating leukocyte count of 1000 mL of blood, the risk of CHD death decreased by 14% *(90)*, although the relative odds for a 2000-mL difference in leukocyte count for development of lung cancer ranged from 1.20 to 1.58 in three different populations *(93)*. The circulating leukocyte count was found to be superior to systolic blood pressure, cholesterol levels, and smoking history and was second only to age as a predictor of mortality *(95)*. It must be emphasized that the increments in circulating leukocyte counts that seemingly predispose to degenerative disease and cancer are relatively modest, because they are within the normal range for apparently healthy adults.

It is interesting, and probably not coincidental, that clinical disorders for which elevated circulating leukocyte counts are predictive are essentially the same as those that have been demonstrated in epidemiological studies to be associated with decreased dietary intake and/or plasma levels of the antioxidant nutrients. This implied mechanistic relationship by which sustained, albeit modest, increases in the numbers and pro-oxidative activities of circulating leukocytes cause depletion of nutritional antioxidants and accelerated onset of degenerative diseases and cancer, as well as decline in pulmonary function, remains to be established. Nevertheless, such a relationship is supported by observations that plasma levels of vitamin C are inversely related to circulating total leukocyte and neutrophil counts in smokers and nonsmokers and positively correlated with plasma vitamin E *(50)*. Plasma levels of β-carotene have been reported to correlate inversely with circulating neutrophil counts in young, asymptomatic cigarette smokers but not in nonsmokers *(96)*.

In the setting of sustained increases in the circulating leukocyte count, chronic, excessive production of reactive oxidants by phagocytes may be linked to pulmonary and cardiovascular damage, as well as carcinogenesis. Increased destruction of antioxidants as a result of elevated numbers and activities of leukocytes may also increase the vulnerability of LDL and DNA to oxidative damage in other cell types simply by depleting blood and tissues of these protective agents. Therefore, the circulating leukocyte count, which has surprising predictive power for future development of degenerative diseases and cancer, may be a primary determinant of optimum intake of antioxidant nutrients.

6. CIRCULATING LEUKOCYTE COUNTS AND RECOMMENDED DAILY INTAKE OF VITAMINS C AND E AND β-CAROTENE

The proposed dynamic, inverse association between the levels of the antioxidant nutrients and the circulating leukocyte count remains to be conclusively established. However, if the relationship is substantiated, it may be possible to use the circulating leukocyte count to formulate optimum intakes of vitamins C and E and β-carotene. We used data from a study conducted on asymptomatic, young male cigarette smokers to calculate "optimum" daily intake of these antioxidant nutrients based on the numbers and pro-oxidative activities of circulating leukocytes. The average increase in the circulating leukocyte count of smokers in our study was 2×10^6/mL (6.2×10^6/ml and 8.2×10^6/mL for 85 male nonsmokers and 100 age- and gender-matched cigarette smokers, respectively). The average increase in the smoking-related phagocyte-mediated oxidant burden was calculated to be 71% (because of increased numbers and pro-oxidative

activities of circulating leukocytes). Based on this increase in the oxidant burden and on Recommended Daily Allowances (RDAs) of 60 mg/d and 15 mg/d (these are not the current RDAs for vitamin C and vitamin E), respectively, and a proposed intake of 6 mg/d for β-carotene, the estimated increases in the daily intakes (above the RDA levels) for each increment of 1×10^6/mL in the circulating leukocyte count above 6.2×10^6/mL are 20 mg of vitamin C, 5 mg of vitamin E, and 2 mg of β-carotene. Given that the upper limit of the normal range for circulating leukocyte counts for apparently healthy adult humans is approx 11×10^6/mL, the estimated daily intakes of the antioxidant nutrients required to protect all individuals with leukocyte counts less than or equal to this value are 160 mg, 40 mg, and 16 mg for vitamin C, vitamin E, and β-carotene, respectively. Admittedly, there are several flaws in the calculation, such as: (a) it is based on RDAs that may or may not be optimal; (b) in vivo activation of phagocytes may not be representative of the in vitro situation; and (c) it may not be necessary to increase the daily intake of all three antioxidants. This may be particularly important in the case of β-carotene, for which a daily intake in excess of 6 mg probably is not justified and may even be hazardous for cigarette smokers *(74)*. However, despite these limitations, it is noteworthy that these values are in good agreement with intake estimates based on data from metabolic *(97)*, experimental *(98)*, and epidemiological studies *(43)*. Modest, daily supplementation above normal dietary intake levels, with 60 mg of vitamin C, 30 mg of vitamin E, and 3 mg of β-carotene in combination with zinc, selenium, and copper for 60 d has been reported to protect nonsmokers against the harmful pro-oxidative effects (DNA damage and lipid peroxidation) of exposure to environmental tobacco smoke in the workplace *(99)*. Until such time as the potential benefits of vitamin supplementation in disease prevention are clearly established or discounted, it has been proposed that it may be prudent for all adults to practice routine, modest supplementation *(100)*.

However, it must be emphasized that several settings exist in which short-term administration of vitamins C and E at supra-RDA levels have clear health benefits for certain subgroups. These include the critically ill *(101,102)*; those with endothelial dysfunction associated with atherosclerosis, hypercholesterolemia, smoking, and hypertension *(72,87)*; and, possibly, those with tuberculosis and/or HIV/AIDS *(103,104)*.

7. CONCLUSIONS

Lipoproteins, extracellular matrix proteins (such as elastin), and many cellular molecules (including DNA, membrane lipids, and enzymes involved in DNA repair and energy production) are extremely vulnerable to oxidant-mediated damage. Therefore, certain avoidable and unavoidable aspects of lifestyle, because they are associated with accelerated destruction and/or decreased intake of antioxidant nutrients, may predispose to development of acquired immune dysfunction and increased risk of infection *(105)*, cancer, and degenerative diseases such as atherosclerosis, cataracts, and pulmonary emphysema.

8. RECOMMENDATIONS

Avoidance of unhealthy aspects of lifestyle, such as low intake of fresh fruits and vegetables, cigarette smoking, and excessive exposure to sunlight, are essential for maintenance of antioxidant homeostasis and prevention of oxidant-mediated diseases.

Because some of these objectives are difficult to achieve, even in affluent societies *(106)*, modest supplementation with antioxidant nutrients represents a potentially efficient and inexpensive strategy to counteract the potential threat of oxidant-inflicted diseases. Daily intakes of about 200 mg, 40 mg, and 6–16 mg of vitamin C, vitamin E, and β-carotene, respectively, seem appropriate.

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21 Nutritional Supplements and Upper Respiratory Tract Illnesses in Young Children in the United States

Linda A. Linday

KEY POINTS

- In the United States, children have lower blood levels than adults of eicosapentaenoic acid (EPA), an important ω-3 fatty acid that helps decrease inflammation; vitamin A, the "anti-infective" vitamin; and selenium (Se), a trace metal that is an intrinsic part of glutathione peroxidase, an important free-radical scavenging enzyme.
- EPA, vitamin A, and Se are important in controlling inflammation and can be supplied by oral nutritional supplements.
- Cod liver oil contains EPA (and other important ω-3 fatty acids), and vitamin A as well as vitamin D.
- Fish oil contains ω-3 fatty acids (including EPA) but no vitamins.
- Our clinical research demonstrates that daily supplementation with a flavored cod liver oil (which meets European purity standards) and a children's multivitamin-mineral with trace metals, including Se, can decrease morbidity from upper respiratory tract illnesses, otitis media, and sinusitis in young children living in the United States.
- These supplements can be used by practitioners on an individual basis, when clinically indicated; the supplements can be purchased in the United States without a prescription.
- Socioeconomically disadvantaged children are at risk for micronutrient deficiencies. However, their families may not be able to afford to purchase these supplements, which are not available through Medicaid, The Special Supplemental Nutrition Program for Women, Infants and Children, or the Food Stamp Program.
- If our results are confirmed in larger studies, a system change will be needed to provide these supplements to nutritionally vulnerable, socioeconomically disadvantaged children living in the United States.

1. INTRODUCTION

Upper respiratory tract illnesses, otitis media (OM), and sinusitis are common and costly disorders among young children living in the United States. Inflammation is a

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key element in the pathophysiology of these disorders. This chapter discusses the role of essential fatty acids, vitamins, and trace metals in the pathophysiology of inflammation; reviews our clinical research on the use of a lemon-flavored cod liver oil (which meets European purity standards) and a children's chewable multivitamin-mineral with Se for the prevention and adjunctive treatment of these disorders; reviews the history of cod liver oil, including its importance in the discovery of vitamin D and the anti-infective properties of vitamin A; and discusses the current clinical use of these supplements. If additional research confirms the utility of these supplements in improving the health of young children, the problem of access to these supplements by socioeconomically disadvantaged children in the United States will need to be addressed.

2. BACKGROUND

2.1. Upper Respiratory Tract Illnesses, Otitis Media, and Sinusitis in Children in the United States

Children under age 5 yr had an average of 20.7 million ambulatory care visits per year for upper respiratory conditions in the United States from 1993 to 1995, with another 14.5 million visits per year for OM during the same time period *(1)*. In addition to their cost, unnecessary health care visits for the treatment of colds generate a significant number of inappropriate antibiotic prescriptions *(2,3)*, although the situation is now improving *(4,5)*. Bacterial antibiotic resistance is considered to be a major public health problem in the United States, and an interagency federal action plan has identified the decrease of unnecessary antibiotic prescriptions as critical to combating antimicrobial resistance *(6)*.

In 1996, Gates *(7)* estimated that the total annual cost of treating OM with effusion in the United States was $5 billion, including both direct and indirect costs. This estimate included the cost of surgical placement of tympanostomy tubes (ventilation tubes placed in the tympanic membrane of the ear), a procedure that is commonly performed for the treatment of this disorder *(8)*. Frequent OM with effusion in early childhood may be associated with later speech and language problems, although causative relationships have not been definitively established *(9,10)*.

Sinusitis also is a common and costly condition *(11)*; in 1996, overall health care expenditures for sinusitis in the United States were estimated to be $5.8 billion, of which $1.8 billion was for children age 12 yr or younger. The primary treatment of sinusitis in children is medical management. Adenoidectomy may be helpful *(12)*, although endoscopic sinus surgery is reserved for chronic, refractory cases *(13,14)*. Chronic sinusitis has a major negative impact on the quality of life of children whose disease is sufficiently severe to require endoscopic sinus surgery *(15)*.

2.2. Viruses and Vaccines

Viral illnesses usually are brief and self-limited conditions. However, viral infections produce inflammation, enhance nasopharyngeal bacterial colonization and adherence, alter the host's immune defenses, and are associated with bacterial complications, including acute OM *(16)*, sinusitis, and pneumonia *(17,18)*. Acute OM occurs in about 20% of children with viral upper respiratory infections *(17)*. Although viral vaccines are in development, the use of vaccines to prevent upper respiratory tract infections is hampered by the large number of different viruses causing these

infections *(17)*. The US Advisory Committee on Immunization Practices voted to recommend influenza vaccination for children ages 6 to 23 mo for the coming influenza season of 2004 to 2005; the impact of this recommendation remains to be seen *(19)*. Despite the fact that *Streptococcus pneumoniae* is the most common cause of bacterial acute OM, the heptavalent pneumococcal polysaccharide conjugate vaccine only produced a 6% reduction in the overall number of episodes of acute OM from any cause *(20)*.

2.3. Free Radicals, Trace Metals, and Inflammation

Free radicals are molecules, or molecular fragments, with one or more unpaired electrons in their outer orbit; they are highly reactive chemical entities that can initiate oxidative stress and damage lipid membranes by a process known as lipid peroxidation. Free radicals are important in the pathophysiology of inflammation; they can also damage proteins and DNA, and have been linked to numerous disease states *(21)*. The body's free-radical scavenging capacity is determined by the levels of free radical scavenging enzymes, as well as their related trace metals and vitamins; this system protects membranes from peroxidation *(21)*. The trace metals Se, zinc, copper, and manganese are critical for the function of these enzymes; oral supplements of trace metals can increase the activity of the free radical scavenging enzymes.

Glutathione peroxidase (GSH-Px) is one of the key enzymes in the protective free-radical scavenging system, and Se is a structural component of the active center of GSH-Px *(22)*. GSH-Px activity decreases during Se depletion and increases during Se repletion *(22–24)*. Superoxide dismutase (SOD) is another important free-radical scavenging enzyme. Two SOD isoenzymes have been identified in mammalian cells. An isoenzyme containing copper and zinc has been found in the cytosol; the SOD isoenzyme found in the mitochondria contains manganese. These isoenzymes are dependent on their respective trace metals for their activity *(24)*. Vitamins, especially vitamins A and C and the B vitamins, are important cofactors in this system. Other free-radical scavenging enzymes include glutathione reductase, glutathione transferase, and catalase, discussed in refs. *24* and *25*.

2.4. Essential Fatty Acids

Fatty acids containing more than one double bond are classified as polyunsaturated fatty acids (PUFAs). There are two series of PUFAs, the ω-3 and ω-6 fatty acids, named for the distance of the first double bond from the methyl end of the molecule *(26)*. The two essential fatty acids (EFAs) are linoleic acid, the parent compound of the ω-6 series, and α-linolenic acid, the parent compound of the ω-3 series. They are essential because humans, like all mammals, cannot make them and must obtain them from their diet. Once consumed, linoleic acid and α-linoneic acid may be elongated further and desaturated to form long-chain PUFAs *(26)*. The ω-3 and -6 series are not interconvertible in the human body; they are metabolically and functionally distinct, compete for the same enzymes, have opposing physiological functions, and their proper balance is important for homeostasis and normal development *(26)*.

EPA and docosahexaenoic acid (DHA) are two important ω-3 fatty acids. The brain and retina are rich in DHA *(27)*, which is necessary for normal visual and neural development (ref. *28*; *see* Chapter 26). EPA is an important precursor of anti-inflammatory eicosanoids, whereas the ω-6 fatty acids (especially arachidonic acid [AA]) are precursors

of inflammatory eicosanoids *(29)*. The level of ω-3 fatty acids and other PUFAs is largely determined by diet, unlike proteins, whose structure is genetically determined *(26,30)*.

Changes in dietary habits in the United States in the last 20 to 30 yr have markedly increased the amount of ω-6 EFA consumed (as in vegetable oils), whereas the amount of consumed ω-3 EFA (as in cod liver oil and fish oil) has decreased *(26,31,32)*. The optimal ratio of ω-6/ω-3 EFA in the diet is 3 to 4:1; in the United States, it is currently 10 to 20:1. This abnormal ratio has been linked with numerous disease states, especially those associated with inflammation. ω-3 fatty acid levels can be increased by eating more fatty fish and by consuming nutritional supplements such as cod liver oil, algal-derived long-chain fatty acids, or fish oils *(26,31,32)*.

2.5. Inflammation in Animal Models of Otitis Media and Sinusitis

Free radical-induced lipid peroxidation may play a role in the acute *(33–35)* and chronic inflammation *(36)* of a guinea pig model of acute, unilateral OM caused by infection with *S. pneumoniae.* Inflammatory mediators have been demonstrated in both experimental and human middle ear effusions; these mediators include leukotrienes and prostaglandins, which are metabolites of AA and are derived from phospholipids in cell membranes. Treatment with specific inhibitors of these mediators has prevented the development of OM in some animal models of OM *(37)*. Free radicals are significant components of the inflammatory response, which is part of the pathophysiology of pneumococcal infections *(38)* and the influenza A virus *(39)*; the latter are important causes of OM. Reactive oxygen species (ROS) *(40)* have also been implicated in sinusitis.

2.6. Nutritional Supplements and Infection

The association between respiratory virus infections and acute OM in children is well-established *(41,42)*. Oral supplementation with EFAs *(43)* and zinc *(44)* has been shown to decrease the incidence of respiratory infections in children. Trace elements (including zinc and Se) have been shown to have important effects on the regulation of immune responses *(45)*. Supplementation with ω-3 fatty acids has been reported to be beneficial in preventing infection in surgical patients *(46)*. The importance of ω-3 fatty acids and trace metals (including zinc and Se) is already recognized in the relatively new field of "immunonutrition" *(47–49)*. The clinical efficacy of antioxidants in the treatment of OM has been reported by two groups of Russian investigators *(50,51)*. Of interest is the work of Ginsburg *(52)*, who proposed that the main cause of tissue damage in infectious and inflammatory conditions is synergistic interactions among ROS, microbial hemolysins, enzymes, and cytokines.

2.7. Anti-Inflammatory Properties of Antibiotics

Antibiotics may have anti-inflammatory actions in additional to their antibacterial effects *(53,54)*. Macrolide antibiotics, effective in the treatment of adults with chronic rhinosinusitis *(55)*, have anti-inflammatory properties that contribute to this effect. However, the overuse of antibiotics is associated with the development of bacterial antibiotic resistance. In Finland, the prevalence of macrolide-resistant group A streptococci diminished after the heavy use of macrolide antibiotics decreased *(56)*. In this age of antibiotic resistance, it is preferable to use antibiotics for their antibacterial effects rather than as anti-inflammatory agents.

3. CLINICAL RESEARCH

3.1. Blood Levels of Fatty Acids, Trace Metals, and Vitamin A

In our first study *(57)*, we obtained blood samples from 44 children undergoing clinically indicated ambulatory surgery at The New York Eye & Ear Infirmary (NYEE). There were 39 subjects in the tympanostomy tube group (TT); these children were undergoing placement of tympanostomy tubes for frequent ear infections and/or persistent middle ear effusion, with or without concomitant adenoidectomy and/or tonsillectomy. Their mean (± standard deviation [SD]) age was 3.8 ± 1.8 yr; 72% were male; approximately half were Hispanic and half were white; approximately half were private patients; and almost half were taking vitamin supplements.

The comparison group (COMP) was composed of children undergoing eye-muscle surgery as well as those undergoing ear, nose, and throat procedures such as bronchoscopy or laryngoscopy that did not involve the ears, adenoids, or tonsils. These subjects were slightly older, with a mean age of 5.7 ± 1.8 yr, and there was a lower percentage (20%) of private patients.

No demographical information was available for the six adults in the adult control group (AC), supplied by Ann Moser of the Peroxisomal Disease Section of the Kennedy Krieger Institute Genetics Laboratory (Baltimore, Maryland). Data regarding red blood cell (RBC) fatty acids, trace metals, and vitamin A were available for subsets of these subjects.

3.1.1. Red Blood Cell Fatty Acids

The RBC fatty acid data are summarized in Table 1. The mean values for EPA were lower in both groups of NYEE children than in the ACs supplied by Kennedy Krieger. The mean RBC EPA values were: (a) TT = 0.31% ± 0.02% (standard error [SE]), (n = 16); (b) COMP = 0.31% ± 0.04% (SE), (n = 5); and (c) AC = 0.48% ± 0.4% (SE), (n = 6). These differences were statistically significant when analyzed by both parametric (ANOVA, $p < 0.002$; F[2,24] = 8.336) and nonparametric (Kruskal-Wallis ANOVA by Ranks, $p = 0.007$; H[2, n = 27] = 9.924) tests. No other significant differences in RBC fatty acids were noted among the three groups, although additional differences might become apparent with larger sample sizes.

In 1986, Japanese investigators reported lower plasma levels of ω-3 fatty acids in young normal and atopic children than in adults *(58)*. They ascribed these findings to dietary changes in Japan in the 30 yr prior to the study.

3.1.2. Trace Metals

Data for plasma Se, zinc, and copper for the children in the TT group are summarized in Table 2; data from the published literature for children *(59)* and adults *(25)* for these parameters is also included, as are International System Units. There was no statistically significant difference between the mean plasma Se for study subjects (TT = 110 ng/mL ± 16.3 SD; n = 39) and the published values for children. Both groups of children had lower Se levels than published values for adults ($p < 0.005$, ANOVA; F = 20.442; F[0.005] 3140 = 4.47) *(60)*. Statistical analyses were performed only for Se because only the variances for Se were homogeneous (Bartlett's test for homogeneity of variances *(60)*.

3.1.3. Vitamin A

The mean plasma vitamin A (retinol) level for the TT group was 39.1 µg/dL ± 10.8 (SD); (range: 23.6–72.1 µg/dL; n = 39). (Multiply conventional units by 0.0349 to convert

Table 1
Red Blood Cell Levels of Fatty Acids

Fatty acids	*Tympanosotomy tube group (NYEE children; n = 16)*	*Comparison group (NYEE children; n = 5)*	*Adult control group[a] (Kennedy Krieger; n = 6)*
ω-6 18:2 ω-6 (linoleic)	9.35 ± 0.28	9.46 ± 0.50	8.55 ± 0.45
20:3 ω-6 (dihomo-γ-linolenic)	1.57 ± 0.08	1.69 ± 0.15	1.70 ± 0.14
20:4 ω-6 (AA)	15.94 ± 0.36	15.44 ± 0.64	16.36 ± 0.59
ω-3 18:3 ω-3 (α-linolenic)	0.29 ± 0.06	0.23 ± 0.10	0.29 ± 0.09
20:5 ω-3 (EPA)	0.31 ± 0.02[b]	0.31 ± 0.04[b]	0.48 ± 0.04
22:6 ω-3 (DHA)	3.78 ± 0.33	3.65 ± 0.60	3.97 ± 0.54
Total ω-6	33.49 ± 0.40	33.38 ± 0.72	32.31 ± 0.66
Total ω-3	6.35 ± 0.36	6.10 ± 0.65	6.79 ± 0.60
Total ω-9	13.56 ± 0.26	13.44 ± 0.47	13.76 ± 0.43

Data are mean percentage of total fatty acids ± SE.

[a]Adult Control data supplied by Ann Moser of Peroxisomal Diseases Section of Kennedy Krieger Institute Genetics Laboratory (Baltimore, Maryland).

[b]Values for NYEE groups are less than those for adult control group; $p = 0.007$, Kruskal-Wallis analysis of variance by ranks.

NYEE, New York Eye and Ear Infirmary; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

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to international units *[61]*). Ballew *(62)* defined an inadequate vitamin A level as less than 20 µg/dL and a suboptimal vitamin A level as less than 30 µg/dL. The upper limit of the pediatric reference range for vitamin A is 80 µg/dL *(61)*. Therefore, overall, the values for our subjects were within the reference range. Russell *(63)* stated that a vitamin A level of 40 µg/dL predicted normal dark adaptation 95% of the time; 69% (27/39) of our subjects had vitamin A levels less than or equal to 40 µg/dL, although parents denied symptoms of night blindness for all children. Our finding is consistent with Ballew's *(62)* report that the 75th percentile for serum retinol levels in children ages 4 to 8 yr was 39.0 µg/dL. In addition, 15% (6/39) of our sample had suboptimal levels (<30 µg/dL), although none were inadequate *(62)*. In this suboptimal group, five of six children were not taking vitamin supplements, five of six children were Hispanic, five of six children were general service patients, and four of six children were female (all of whom were Hispanic). The overrepresentation of Hispanic children in the subgroup of children with suboptimal vitamin A levels was also consistent with previous reports *(62)*.

On a group basis, there was no statistically significant difference in the mean vitamin A levels between the subgroup of children whose families reported that they were taking vitamin supplements (39.7 µg/dL ± 11.6 SD; $n = 17$) and the subgroup of children whose families reported that they were not taking vitamin supplements (38.7 µg/dL ± 10.4 SD; $n = 22$). Our subjects took a variety of prescription and over-the-counter vitamin preparations; no children were receiving or had taken cod liver oil; only one child in the fatty acid subgroup had a history of fish oil ingestion. Of the eight different children's vitamin preparations examined, all contained vitamin A palmitate, vitamin A acetate, and/or β-carotene.

Table 2
Plasma Levels of Selenium, Zinc, and Copper

		Tympanostomy tube group (57) (NYEE Children; n = 39)	*Children (59) (n = 83)*	*Adult Long Island Jewish Controls (25) (n = 12)*	*Adult West Coast Controls (25) (n = 14)*
Selenium	(ng/mL)	110 ± 16[a]	106 ± 18[a]	129 ± 22	142 ± 18
	(μmol/L)	1.40 ± 0.21	1.35 ± 0.23	1.64 ± 0.27	1.81 ± 0.23
Zinc	(μg/mL)	0.9 ± 0.1	1.0 ± 0.2	1.3 ± 0.2	1.8 ± 0.5
	(μmol/L)	13.9 ± 2.0	15.3 ± 3.1	19.9 ± 3.1	27.5 ± 7.6
Copper	(μg/mL)	1.3 ± 0.2	1.2 ± 0.3	1.1 ± 0.2	1.3 ± 0.5
	(μmol/L)	20.7 ± 3.3	18.8 ± 4.7	17.3 ± 3.1	20.4 ± 7.8

Data are mean ± SD.
[a]Children less than adults ($p < 0.005$, analysis of variance).
(Reprinted with permission from ref. *57*.)

3.1.4. Summary of Blood-Level Data and Choice of Nutritional Supplements

The blood-level data *(57)* revealed that (a) study children had lower levels of RBC EPA than adult controls; (b) 69% of study children had plasma vitamin A (retinol) levels in the lower reference range, with 15% in the suboptimal range; and (c) study subjects, like other children, had lower levels of plasma Se than adults.

Therefore, for our clinical studies, we chose cod liver oil as a source of both vitamin A and EPA and used it in conjunction with a marketed children's chewable multivitamin/mineral preparation containing Se. We specifically chose cod liver oil as a source of vitamin A on the basis of our blood-level data, which revealed no significant difference in the vitamin A levels between the subgroups of children who were taking vitamin supplements and those who were not. Although we could have used fish oil as the source of EPA, fish oil does not contain vitamin A or D. The detailed contents of these supplements are shown in Table 3. The vitamin A content of cod liver oil used in our initial pilot study on OM was 2000 to 2500 IU/teaspoon (5 mL); in the subsequent two studies, the vitamin A content was decreased to 1000 to 1250 IU/5 mL.

3.2. Pilot Clinical Research on Otitis Media

To explore the clinical utility of these supplements, we then performed an open-label secondary prevention study, in which each child served as his or her own control *(64)*. We studied one OM season, from September 1, 2000 to March 31, 2001 *(57)*. All children were patients of the Soho Pediatrics Group, a private group practice in lower Manhattan, New York. Children were required to have had at least one episode of OM from September 1 to November 30, 2000 (the early portion of OM season under consideration). Children with a known allergy to fish were excluded.

Eight children were enrolled, ranging in age from 0.8 to 4.4 yr; seven were Caucasian, half were female, and all families were English-speaking. After enrollment, subjects received 1 teaspoon of lemon-flavored Norwegian cod liver oil and one-half of a tablet of Carlson's Scooter Rabbit chewable multivitamin-mineral (MVM) tablet per day (*see* Table 3). Mothers were instructed to crush the MVM

Table 3
Contents of Supplements

Carlson's lemon-flavored cod liver oil (1 teaspoon; 5 mL):	
ω-3 essential fatty acids:	
DHA	500–550 mg
EPA	460–500 mg
α-Linolenic acid	45–50 mg
Vitamins:	
Vitamin A	1000–1250 IU: randomized pediatric sites *(83)* 1000–1250 IU: Sinusitis Pilot Study *(81)* 2000–2500 IU: Otitis Media Pilot Study *(57)*
Vitamin D	400–500 IU
Vitamin E	1 IU
One-half of a tablet of Carlson's Scooter Rabbit chewable vitamins and minerals[a]:	
Vitamins:	
Vitamin A (palmitate)	2500 IU
Vitamin D_3	200 IU
Vitamin E	30 IU
Vitamin K	20 µg
Vitamin C	60 mg
Thiamin (B_1)	0.75 mg
Riboflavin (B_2)	0.85 mg
Niacin	2.5 mg
Vitamin B_6 (pyridoxine)	1.0 mg
Folate (folic acid)	100 µg
Vitamin B_{12} (cyanocobalamin)	3 µg
Biotin	15 µg
Pantothenic acid	5 mg
Minerals:	
Calcium	25 mg
Iron	4.5 mg
Phosphorus	11 mg
Iodine	37.5 µg
Magnesium	12.5 mg
Zinc	3.75 mg
Selenium	17.5 µg
Copper	0.5 mg
Manganese	0.88 mg
Chromium	30 µg
Molybdenum	19 µg
Potassium	1.25 mg
Lemon bioflavenoids	5 mg (daily value not established)

[a]Equivalent to one tablet of Carlson's new Mini Scooter Rabbit chewable vitamins and minerals. (From ref. *57*.)

tablet, measure the cod liver oil, and mix both in a small amount of food (such as applesauce, yogurt, or rice cereal) to administer the supplements to their children. Parents were informed verbally and in writing that supplements were to be given only in the amounts required by the study and that study supplements were to be kept out of reach of children.

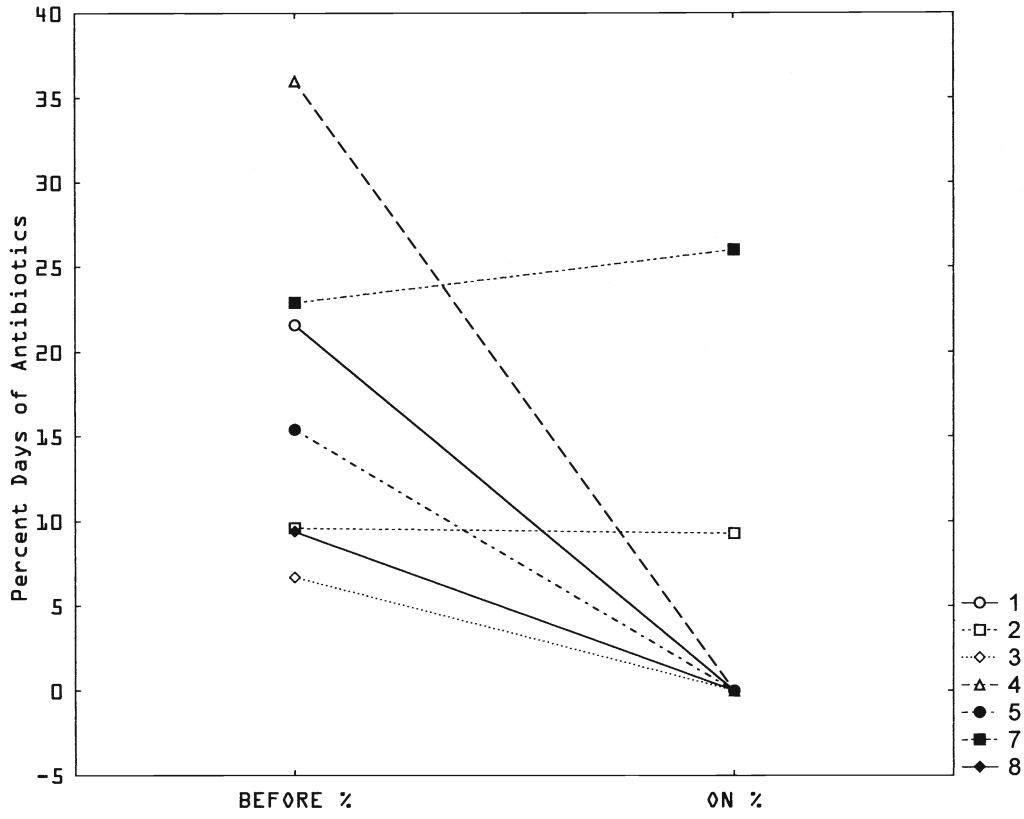

Fig. 1. Otitis Media Pilot Study: percent days of antibiotics. Subjects received antibiotics for OM for 12.3 ± 13.4 (SD) fewer days during supplementation (ON%) than before (BEFORE%); ($p < 0.05$, Wilcoxon Matched Pairs Test). (Reprinted with permission from ref. *57*.)

Of the eight children who entered the study, one could not tolerate the taste of cod liver oil. The remaining seven children received antibiotics for OM for 12.3 ± 13.4 ($p < 0.05$; mean ± SD) fewer days during supplementation than before supplementation during the OM season under study (*see* Fig. 1). Five of seven subjects had no additional episodes of OM during supplementation, although it had no apparent effect on established serous middle ear effusions in two children. However, because our study lacked a randomized, parallel control group, we could not exclude the possibility that the decreased antibiotic usage we found might have occurred without the use of study supplements.

3.3. Effect of these Supplements on Upper Respiratory Pediatric Visits by Young, Inner-City, Latino Children: Randomized Pediatric Sites

Based on our prior research (discussed in ref. *57*) and the historical studies on cod liver oil and upper respiratory illnesses *(65)*, we hypothesized that use of the study supplements by young children would decrease their doctor visits for upper respiratory illnesses during the late fall, winter, and early spring. We studied the effect of daily use of these supplements on the number of pediatric visits by young, inner-city, Latino children from late autumn 2002 to early spring 2003 *(83)*.

We did not have a matched placebo for liquid cod liver oil. Although adults and older children can swallow capsules, infants and toddlers cannot. Furthermore, if capsules were cut open to administer the contents, the distinctive odor and taste of cod liver oil would immediately become apparent *(57)*.

The absence of a matching placebo for liquid cod liver oil precluded our performing a classical double-blind, placebo-controlled study. Lack of a placebo coupled with the fact that cod liver oil can be purchased without a prescription by interested parties *(57,66)* led us to choose a study design in which we randomized pediatric sites, rather than individual patients. This type of design has been used in the worldwide studies of vitamin A supplementation, which are discussed in Subheading Section 5.3, that have included randomization by ward, household, village, or district *(67)*. It was also used in a food-consumption study to avoid changes in food habits resulting from knowledge of the other treatment *(68)*. Randomized site design is commonly used in behavioral and educational studies where no placebo is possible *(69–72)*; a recent study of herd immunity and the pediatric heptavalent pneumococcal vaccine also used a randomized site design *(73)*. To minimize the influence of the study on the behavior of the participating families *(69)*, we used a "no-contact" control group *(74,75)*, which has also been used in behavioral and educational studies.

The study was performed at Pediatrics 2000, a multisite, private, pediatric group practice in New York City. Two of the offices with similar demographics (low-income Latino families), located 1.1 miles apart in upper Manhattan, were randomized to a supplementation site and a medical records control site. Study participants were children ages 6 mo to 5 yr of either gender and any race, religion, or nationality who were patients enrolled at the two offices where the study was being performed. Study participants were required to be in New York City from enrollment through April 2003 (with the exception of brief vacations), and to have some type of medical insurance. Patients who routinely received additional health care at other practices or medical centers were excluded; children with known fish allergy, a chronic, life-threatening condition (such as HIV/AIDS or cancer), feeding disorders, and epilepsy were also excluded. Study materials were available in both English and Spanish. Per practice routine, two professional coders reviewed all charts from both sites, coded the visits, and entered the data into a computer with NDC Medisoft™ Network Professional 7.02 software *(76)*.

Participants in the medical records control group were enrolled from October 21 to November 10, 2002; those in the supplementation group were enrolled from November 11 to December 12, 2002. We were unable to randomize enrollment at the two sites because the lemon-flavored cod liver oil used in the study (which was manufactured in Norway) had been reformulated with less vitamin A *(77)* and was delayed in the US Customs Office. The study follow-up/supplementation period ended on May 1, 2003.

A total of 94 children (47 at each site) were enrolled in the study. The mean age of the supplementation group was 2.03 yr (±1.04 SD), and the mean age of the control group was 2.08 yr (±1.10 SD). There were no statistically significant differences in the demographical characteristics of the study participants in the two groups: most were Latino children from low-income families (as indicated by health insurance), and their mothers were predominantly unmarried immigrants from the Dominican Republic whose first language was Spanish.

Children of at least 1 yr of age received 1 teaspoon of Carlson's lemon-flavored cod liver oil per day and one-half tablet of Carlson's Scooter Rabbit chewable MVM, the same doses used in our previous research administered in the same manner *(57)*.

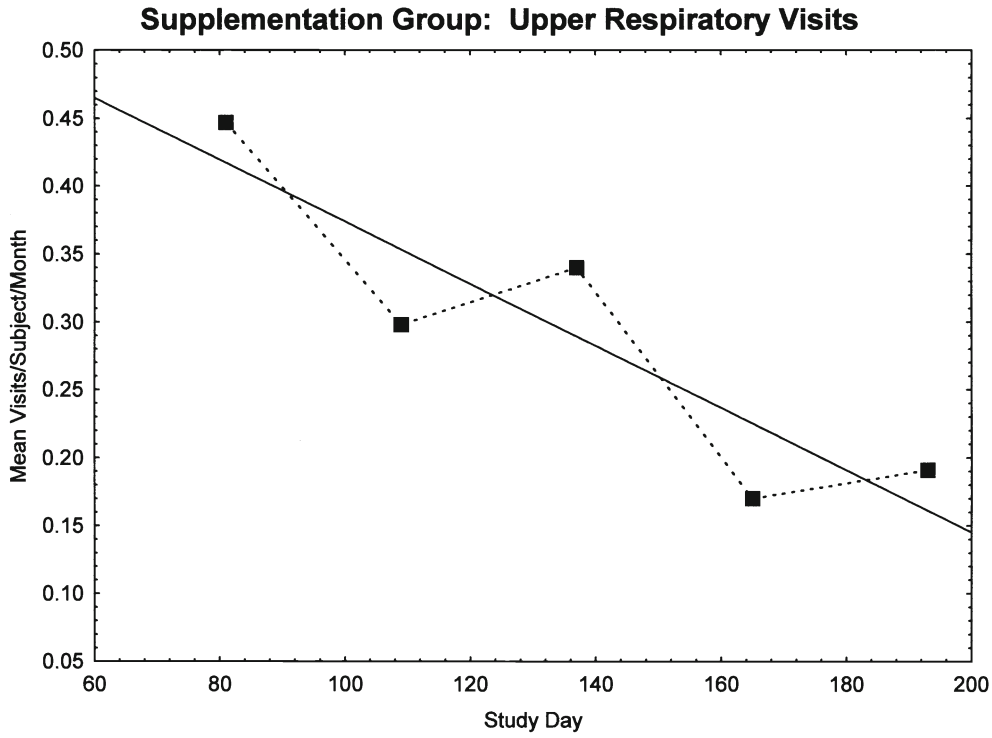

Fig. 2. Randomized pediatric sites: the supplementation group had a statistically significant decrease in the mean number of upper respiratory visits over the course of the follow-up/supplementation period ($p = 0.042$; $r = 0.893$; $r^2 = 0.797$; $y = 0.602 - 0.002x$). (Reprinted with permission from ref. *83*.)

However, the vitamin A content of the cod liver oil was approximately half that used in our first pilot study of OM (see Table 3). Thus, the full dose of supplements provided a total of 3750 IU of vitamin A and 700 IU of vitamin D per day. However, in the current study, the starting dose of supplements was halved for children ages 6 mo to 1 yr .Visits were classified as upper respiratory visits, other illness visits, or visits not analyzed on the basis of the ICD-9 visit code *(78)*. The primary outcome measure was upper respiratory visits during the follow-up/supplementation period; other illness visits during the same time period were considered as secondary outcome measures.

As shown in Figs. 2 and 3 and Table 4, the supplementation group had a statistically significant decrease in the mean number of upper respiratory visits over the course of the follow-up/supplementation period ($p = 0.042$; $r = 0.893$; $r^2 = 0.797$; $y = 0.602 - 0.002x$), whereas the medical records control group had no change in this parameter ($p = 0.999$; $r = 0.0006$; $r^2 = 0.0000$; $y = 0.259 + 1.43 \times 10^{-6}x$). There was no statistically significant change in the mean number of other illness visits for either study group during the same time period. Although there was a significant difference in the pattern of decreasing upper respiratory visits over time in the supplementation group, there was no difference in the total number of visits made by the two groups. Data were analyzed on an intention-to-treat basis.

As reported by their parents, 70% of our subjects completed a 5- to 6-mo course of lemon-flavored cod liver oil. By comparison, only 47% of families reported compliance

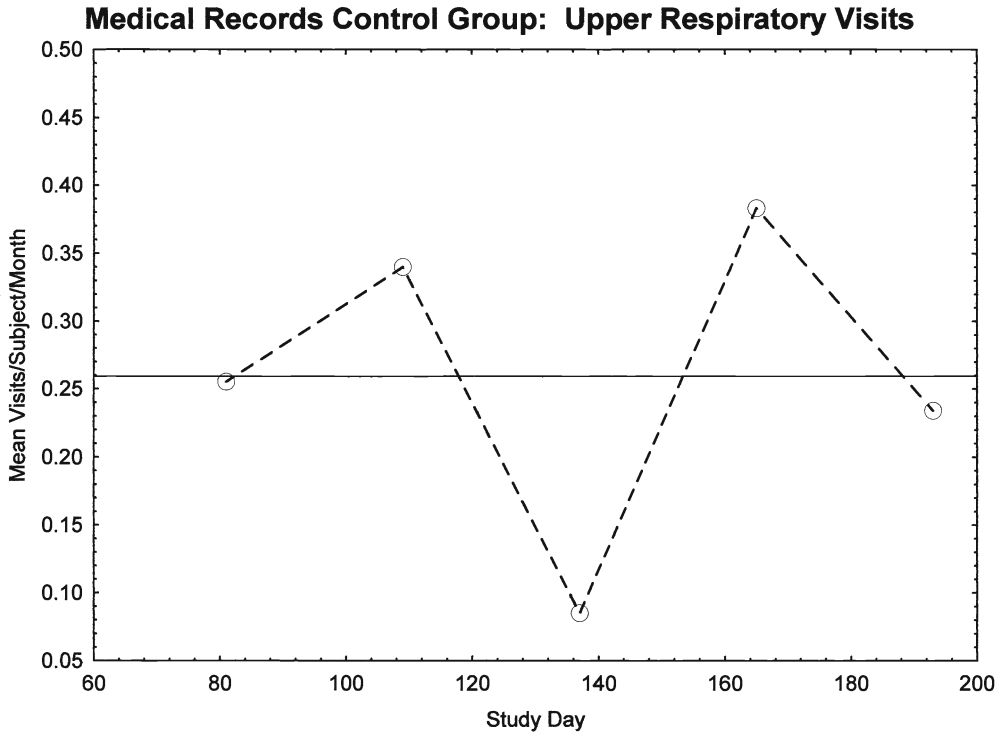

Fig. 3. Randomized pediatric sites: the medical records control group had no change in the mean number of upper respiratory visits over the course of the follow-up/supplementation period ($p = 0.999$; $r = 0.0006$; $r^2 = 0.0000$; $y = 0.259 + 1.43 \times 10^{-6}x$). (Reprinted with permission from ref. *83*.)

with antibiotic prophylaxis for OM in a study of Latino children who attended an otolaryngology clinic *(79)*. Our favorable compliance rates may partly result from the fact that young children in the Dominican Republic are often given cod liver oil or similar supplements, although families rarely continue this practice after moving to the United States.

3.4. Adjunctive Therapy for Children With Chronic/Recurrent Sinusitis: Pilot Research

Inflammation and edema of the sinonasal mucosa are important in the pathophysiology of sinusitis. Based on our previous research and the similarities between OM and sinusitis *(80)*, we hypothesized that these nutritional supplements would also be effective adjunctive therapy for the treatment of children with chronic and/or recurrent sinusitis. Therefore, we performed a 4-mo, open-label, dose-titration study in which each patient served as his or her own control *(64,81)*.

Study participants were private pediatric otolaryngology outpatients of Jay N. Dolitsky, MD, who resided in the New York Metropolitan area and had a clinical diagnosis of chronic and/or recurrent sinusitis as well as symptoms of at least 3 mo of duration that were refractory to treatment with antibiotics. Subjects were between ages 2 and 18 yr, of either gender and any race, religion, or nationality. Children with known allergy to fish; chronic, life-threatening condition (such as HIV/AIDS or cancer); feeding disorder;

Table 4
Randomized Pediatric Sites: Upper Respiratory Visits and Other Illness Visits During the Follow-Up/Supplementation Period

		Upper respiratory visits				*Other illness visits*			
		Supplementation group (n = 47)		*Medical records control group (n = 47)*		*Supplementation group (n = 47)*		*Medical records control group (n = 47)*	
Dates	*Study days*	*No. of visits*	*Mean visits/subject*	*No. of visits*	*Mean visits/subject*	*No. of visits*	*Mean visits/subject*	*No. of visits*	*Mean visits/subject*
12/13/2002 to 1/9/2003	54–81	21	0.45	12	0.26	3	0.06	0	0
1/10/2003 to 2/6/2003	82–109	14	0.30	16	0.34	6	0.13	3	0.06
2/7/2003 to 3/6/2003	110–137	16	0.34	4	0.09	6	0.13	2	0.04
3/7/2003 to 4/3/2003	138–165	8	0.17	18	0.38	2	0.04	3	0.06
4/4/2003 to 5/1/2003	166–193	9	0.19[a]	11	0.23[b]	1	0.02[c]	2	0.04[d]

[a] $p = 0.042$; $r = 0.893$; $r^2 = 0.797$; $y = 0.602 - 0.002x$
[b] $p = 0.999$; $r = 0.0006$; $r^2 = 0.0000$; $y = 0.259 + 1.43 \times 10^{-6}x$
[c] $p = 0.337$; $r = -0.550$; $r^2 = 0.303$; $y = 0.160 - 0.61 \times 10^{-3}x$
[d] $p = 0.369$; $r = 0.520$; $r^2 = 0.271$; $y = 0.72 \times 10^{-3} + 0.31 \times 10^{-3}x$
(Reprinted with permission from ref. *83*.)

seizure disorder; known cystic fibrosis; aspirin-intolerant asthma; and family plans to move outside the metropolitan area during the course of the study were excluded. Subjects were enrolled from late January to early March 2003 and received supplements for 4 mo from the time of enrollment. Primary endpoints were the number of doctor visits for acute respiratory illnesses and the child's sinus symptoms, which were quantified using a pediatric sinusitis symptom questionnaire *(82)*.

The starting dose of supplements in the current study was the same as in our previous research *(57)*: 1 teaspoon (5 mL) of Carlson's lemon-flavored cod liver oil and one-half of a tablet of Carlson's Scooter Rabbit chewable multivitamin-mineral per day (providing a total of 3750 IU of vitamin A and 700 IU of vitamin D per day). The vitamin A content of the cod liver oil was lower than in our first pilot study of OM *(57)* but was the same as that used in our study of Latino children (*83*; *see* Table 3). Supplement doses could be doubled to an intermediate dose (providing 7500 IU of vitamin A and 1400 IU of vitamin D per day) within 2 to 3 wk . If higher doses were needed, cod liver oil was discontinued and fish oil was administered instead (fish oil does not contain vitamin A or D). The maximum dose of fish oil was 3 g/d, and the maximum dose of multivitamin-minerals was four half-tablets per day (providing 10,000 IU of vitamin A and 800 IU of vitamin D per day). The titrated doses of vitamins A and D were higher than those used in our previous study *(57)* but were well-below the lowest daily toxic doses of these vitamins *(61)* (19,860 IU/d for vitamin A and 2800 IU/d for vitamin D). The US Food and Drug Administration (FDA) considers fish oil at dosages up to 3 g per day as safe for adults and children *(84)*.

Our four subjects were Caucasian males, ranging in age from 4.2 to 9.8 yr, with chronic/recurrent sinusitis for at least 3 yr prior to entry in the study. Three subjects had a positive response; one subject dropped out for administrative reasons. The responders had decreased sinus symptoms, fewer episodes of acute sinusitis, and fewer doctor visits for acute illnesses at 4, 6, and 8 wk after beginning study supplements. Their parents reported that they had begun to recover from upper respiratory illnesses without complications, which was unusual for these children, as was improvement in springtime; their improvement had previously been limited to the summer months or periods of home-schooling.

Our findings are consistent with prior work by other clinical investigators. In a study of upper respiratory tract infections in young children, Wald and colleagues *(85)* noted that an inflamed respiratory mucosa may not completely recover between episodes of infection. Parsons *(86)* hypothesized that inflammation and edema of the sinonasal mucosa was the primary event in sinusitis, with bacterial infection as a secondary phenomenon.

Chronic/recurrent sinusitis is a debilitating disorder that may require treatment with intravenous antibiotics and/or endoscopic surgery. Use of these supplements as adjunctive therapy for children with chronic/recurrent sinusitis is an inexpensive, noninvasive intervention that clinicians can use for selected patients, pending the outcomes of definitive, large, well-controlled studies.

3.5. Safety

In the 1930s, during the pre-antibiotic era, lipoid aspiration pneumonia was reported with cod liver oil, mineral oil, and egg yolk, which were used at that time to treat sick and debilitated infants *(87)*. In 1950, Caffey *(88)* reported vitamin A toxicity in children who were mistakenly treated with high-dose, long-term vitamin A administered in highly concentrated fish liver oil preparations that were available at that

time. However, none of Caffey's patients had received cod liver oil, and the highly concentrated fish liver oil preparations they received are no longer available in the United States.

In our clinical studies, parents were instructed to crush the half-tablet of MVM, measure the cod liver oil, and mix both with a small amount of food (such as applesauce, yogurt, or rice cereal) before administering the supplements to their child. Additionally, parents were informed both verbally and in writing that supplements were to be given only in the amounts required by the study and that study supplements were to be kept out of reach of children. The principal investigator spoke Spanish, and all parental study materials were available in both Spanish and English. To date, we have not encountered problems with aspiration or overdose in our studies.

3.6. Implications for Asthma

There is a clear association between viral respiratory infections and acute exacerbations of asthma in both children and adults *(17)*. There is also a link between sinusitis and asthma *(89,90)*, with rhinovirus infections linked to both sinusitis and exacerbations of asthma *(90)*. Additionally, Latino children have a high incidence of asthma *(91)*. In view of the results of our studies *(57,81,83)*, we believe that these supplements could be clinically useful for young children (particularly Latino children) with asthma, and we are currently beginning to organize research in this area.

3.7. Nutritional Supplements for Socioeconomically Disadvantaged Children in the United States

Similarly to other countries worldwide *(92)*, socioeconomically disadvantaged children in the United States are at risk for micronutrient deficiencies *(57,62,93)*. Although the supplements used in our research can be purchased in the United States without a prescription, their cost may pose an excessive financial burden to low-income families.

Cod liver oil does not have a National Drug Code number, it is not available through Medicaid in New York, and the children's vitamins we have located that are available through this system do not contain Se or other trace metals. Additionally, cod liver oil is not available through the United States Department of Agriculture (USDA) Special Supplemental Nutrition Program for Women, Infants and Children (WIC); our request for such availability can be found online at http://www.fns.usda.gov/wic/anprmcomments/ihp-06.pdf. Furthermore, purchase of vitamins with US food stamps is not permitted (see http://www.fns.usda.gov/fsp/faqs.htm#9). If our results are confirmed in larger studies, a system change will be required to provide these supplements to nutritionally vulnerable, socioeconomically disadvantaged children living in the United States.

4. HISTORICAL PERSPECTIVE

4.1. History of Cod Liver Oil

Egyptian and Greek physicians may have understood the value of liver (high in vitamin A) for the treatment of night blindness, an early ocular manifestation of vitamin A deficiency *(94,95)*. The use of fish oils in medicine was mentioned by Hippocrates, and Pliny discussed the use of dophin liver oil for the treatment of chronic skin eruptions *(96)*. However, these classical physicians did not appear to know about the use cod liver oil.

The coastal fishermen of northern Europe apparently used cod liver oil for many years for the treatment of aches and pains *(96,97)*. However, the first recorded use of cod liver oil by physicians was from the Manchester Infirmary in England during the 1780s *(96,97)*, where it was found to be very effective for "old pains" and "rheumatism," which were probably cases of osteomalacia (a bone disease of adults) *(96)*. The pattern of discovery was that the use of cod liver oil by fishing folk and peasants was accidentally observed by a physician, who then tried it and made it known to the medical profession *(96)*.

Guy *(96)* states that there was no further mention of cod liver oil in the English medical literature until its revival in 1841 by Bennett, who had observed its use in Germany. Bennett reported that in Holland, cod liver oil had obtained a wide reputation as a cure for rickets (a bone disease of children) "long before its remedial properties were acknowledged by physicians" (*see* ref. *97*, p. 67). In the 1820s, Schenk and Schuette published independent reports in the German literature regarding the value of cod liver oil for curing rickets *(97)*, and Schuette reported that he used cod liver oil successfully for 25 yr. Cod liver oil for the treatment of rickets was introduced in France by Trousseau in the 1830s *(97)*. The demand for cod liver oil was so great that all types of substitutes were used, and reports of failure, contamination, and substitutes for cod liver oil began to appear in the literature before the middle of the 19th century.

4.2. The Discovery of Vitamin A

Vitamin A was discovered as the result of a long, incremental process with contributions by numerous investigators *(95,98)*. At the end of the 19th century and the beginning of the 20th century, nutritional theories were tested under well-controlled laboratory conditions through the administration of experimental diets to animals, and specific factors necessary for their growth and survival began to be identified. During this time, Frederick Hopkins, at Cambridge University, proposed that there were "accessory factors" in foods that were necessary for life but that had not been previously identified; Casimir Funk named these factors "vital amines" or "vitamines" *(65)*.

In 1913, in the same issue of the *Journal of Biological Chemistry*, two groups independently reported the existence of a fat-soluble factor that was essential for the growth of rats *(65,99–101)*. McCollum and Davis of the University of Wisconsin *(99)* demonstrated that after a certain age, the growth of rats was dependent on an ether extract from eggs or butter. Using a different experimental diet, Osborne and Mendel *(100)*, of Yale University, found that there was an "essential accessory factor" in butter needed for the normal growth of rats. This fat-soluble growth factor, originally termed "fat-soluble A," soon became known as "vitamine A" *(65)*.

4.3. The Discovery of Vitamin D

The discovery of vitamin D was closely tied to work on the prevention and treatment of rickets. During the Industrial Revolution, rickets spread rapidly throughout Europe, particularly among the urban poor, who lived in the sunless alleys of factory towns and urban slums *(97)*.

In 1918, Mellanby *(102)*, an English physician and professor of pharmacology, reported the first animal model of rickets, which he developed in puppies. In a simple, two-page report to the Physiological Society, he noted that the daily administration of

foods such as butter, cod liver oil, or 500 cc of milk (among others) was effective in preventing rickets in his model, whereas casein and linseed oil were among the substances that were ineffective. Mellanby felt that rickets was a deficiency disease and stated that "the anti-rachitic accessory factor has characters related to the growth accessory factor [vitamin A], although it is not identical with the latter …" (ref. *102*, p. xi). However, Mellanby was not able to distinguish these two factors; this was accomplished by McCollum and his new collaborators at Johns Hopkins University.

In the 1920s, McCollum and his colleagues developed a rat model of rickets that could also be cured with cod liver oil. They were then faced with the same question that perplexed Mellanby: Was the anti-rachitic factor vitamin A, or was it another substance with a similar distribution as fat-soluble vitamin A (103)? It was known that the vitamin A-deficient animals in these studies often developed ocular abnormalities, including dryness of the eyes, corneal ulceration, and blindness, similar to xeropthalmia in humans *(65)*. Additionally, Hopkins demonstrated that oxidation destroyed fat-soluble A *(103)*. Using these facts, in 1922, McCollum and his colleagues *(104)* reported that when cod liver oil was oxidized for 12 or 20 h, it could no longer cure xerophthalmia, although it could prevent rickets. Therefore, they concluded that the anti-xerophthalmic and the anti-rachitic properties were a result of two distinct substances, and that the anti-rachitic factor, which specifically regulated bone metabolism, was the more heat-stable factor. Because this was the fourth vitamin to be discovered, McCollum's group named it vitamin D in 1925 *(97)*.

The fact that both exposure to sunlight and cod liver oil could prevent or cure rickets was perplexing and controversial *(105)*. Careful experiments by Chick and coworkers *(103)*, working in Vienna from 1919 to 1922, confirmed the value of both cod liver oil and sunlight in the prevention and treatment of rickets in young infants.

In 1919, Huldschinsky *(97)*, a pediatrician in Berlin, used light from a mercury-vapor quartz lamp (which includes ultraviolet [UV] wavelengths) to cure four cases of advanced rickets in children with up to 2 mo of treatment. When Huldschinsky exposed one arm of a rachitic child to the UV irradiation, he found that the rickets in the child's other arm was cured to the same degree as in the exposed arm. Therefore, he concluded that phototherapy was not a local effect and speculated that as a result of exposure to UV light, something was formed in the skin that was then carried to other sites, where it had its anti-rachitic effect *(105)*. In 1925, Hess and Weinstock *(97)* reached similar conclusions based on experimental work in animals. These theories were confirmed in 1936, when Windaus, working in Germany, demonstrated that skin contains the natural prehormone of vitamin D, which is converted to vitamin D_3 when the skin is exposed to UV irradiation (including light from a mercury-vapor lamp) *(97)*.

4.4. Rickets and Respiratory Diseases

Historical investigators were well-aware of an association between rickets and respiratory diseases. In their 1917 paper on rickets, Hess and Unger stated that "rickets is a predisposing cause of these respiratory diseases (pulmonary tuberculosis, pneumonia, and whooping cough)" (ref. *106*, p. 1583). In her 1927 paper on community control of rickets, Eliot stated that "susceptibility to upper respiratory infections, such as colds,

bronchitis and pneumonia, is greatly increased in infancy and early childhood by rickets" (ref. *107*, p. 114). Based on prior animal studies and clinical work by German investigators, Ellison discounted the contribution of vitamin D in the efficacy of cod liver oil for measles. Nonetheless, he acknowledged that "it is possible that some adjuvant effect was obtained from the co-operation of the two factors [vitamins A and D]" (ref. *108*, p. 710). In a 1936 study of vitamins A and D (individually or combined) for children hospitalized with measles, Mackay noted "there is much to indicate that resistance to infections is reduced in children suffering form an overt deficiency of either of these vitamins [vitamin A or D]" (ref. *109*, p. 127).

4.5. Cod Liver Oil for Tuberculosis

Semba noted that cod liver oil, a rich source of vitamins A and D, was used as a treatment for tuberculosis for more than 100 yr *(110)*. In the 1840s, Charlotte Brontë, the author of *Jane Eyre*, suffered from tuberculosis, and her treatment included cod liver oil *(111)*. A 1917 textbook on tuberculosis, although recognizing that there was no specific treatment for tuberculosis at that time, stated that "one of the oldest and best established remedies for the treatment of tuberculosis is cod liver oil" (ref. *112*, p. 467); however, the mechanism of action of cod liver oil was unknown. The situation had changed little by 1946, when Goldberg's textbook stated that cod liver oil "has been used empirically for many centuries in the treatment of pulmonary tuberculosis without any definite knowledge of its action" (ref. *113*, p. C-81). However, the use of cod liver oil for tuberculosis faded as specific treatments were developed, and "cod liver oil" is not listed in the index of a modern textbook on tuberculosis *(114)*.

4.6. Historical Research on the Anti-Infective Properties of Vitamin A

Mellanby (*see* Section 4.3.) had a large colony of dogs that were maintained on experimental diets. In 1926, Mellanby reported, "at one period in the course of my experimental investigations on dogs, the work was greatly hampered by the development of an inflammatory condition of the lungs" (ref. *115*, p. 518), which was bronchopneumonia. On postmortem examination, the pneumonia was largely restricted to the vitamin A-deficient dogs, and he speculated that this might be relevant to respiratory illness in children *(65,115)*. In 1928, Green and Mellanby reported that a deficiency of vitamin A, but not vitamin D, caused increased infections in a rat model, leading them to call vitamin A an "anti-infective" agent; they speculated that this was related to the epithelial changes caused by vitamin A deficiency *(116)*.

In 1932, Ellison *(65,108)* reported the results of a study of concentrated cod liver oil for children who were hospitalized with measles. Ellison was aware of Mellanby's work on the anti-infective properties of vitamin A and also knew that vitamin A deficiency damaged epithelial cells in the respiratory tract *(117,118)*. Ellison specifically chose to study measles because it was "a disease which attacks epithelial defences and whose incidence is greatest in those members of the community who are most likely to be suffering from various grades of vitamin deficiency…the children of the poorest classes" (ref. *108*, p. 709). He studied 600 children under age 5 yr who were admitted to the Grove Hospital (London) with measles. The cases were randomized by ward to treatment with a highly concentrated cod liver oil preparation or a control treatment of standard treatment (no placebo was used). Treatment with cod liver oil reduced measles mortality

by approximately one-half, from 8.7% in the control group to 3.7% in the treated group *(65,108)*. Based on animal studies and German clinical work, Ellison attributed the efficacy of cod liver oil to vitamin A, although he did concede that some adjuvant effect could have been obtained from the cooperation of the two factors (*108*).

A subsequent study published in 1936 *(65,109)* reported that neither vitamins A and D together nor vitamin D alone had an effect on reducing the mortality rate from measles. However, the control mortality rate in this later study decreased to 2.6%, making it difficult to demonstrate an improvement.

By 1940, numerous studies had been conducted to evaluate the ability of vitamin A (usually given as cod liver oil) to decrease the incidence of respiratory infections. The results were mixed, with about half showing a positive impact and the rest demonstrating no effect *(65)*. However, cod liver oil did have a significant impact on decreasing industrial absenteeism *(65)*. In a 1935 study of cod liver oil for the prevention of the common cold in school children, the investigator was not able maintain a control group given no supplements because enthusiastic families purchased cod liver oil for their children outside of the study *(65,66)*; this finding is relevant to our current work.

With the introduction of sulfa antibiotics and penicillin in the 1930s to 1940s *(65)*, as well as the improvements in diet in industrialized countries in the late 1930s, interest in anti-infective therapy shifted to antibiotics and away from vitamin A *(98)*.

4.7. Fortification of Milk With Vitamin D and Synthetic Vitamin A

In the mid-1920s, UV radiation of food and a variety of other substances was demonstrated to produce anti-rachitic properties *(105)*. Steenbock patented the addition of provitamin D to foods followed by UV irradiation to produce anti-rachitic activity. In the 1930s, the addition of provitamin D_2 to milk followed by UV irradiation was widely practiced in the United States and Europe. Rickets was eradicated as a significant public health problem in the countries that used this vitamin D fortification process *(105)*.

In the late 1940s, Otto Isler and his collaborators in Basel reported the synthesis of all-*trans*-vitamin A from the inexpensive precursor β-ionone *(95)*. In the same time period, Arens and van Dorp *(94)* reported the synthesis of retinoic acid. Within a few years, the price of vitamin A fell 10-fold, and it became economically feasible to add vitamin A more generally to foods.

4.8. Cod Liver Oil in the United States

During the latter part of the 19th century, cod liver oil was rarely used in America, although the reason for this lack of use is not clear *(96,97)*. However, there was a resurgence in interest, and in 1917, Hess and Unger wrote, "For many years cod liver oil has been regarded as the sovereign remedy for rickets" (ref. *106*, p. 1583). They successfully prevented rickets with cod liver oil in susceptible African-American babies in a low-income neighborhood in New York City *(106,119)*. Hess urged officials to dispense cod liver oil at the baby health stations at cost, but they declined because it would be too expensive, and they thought that additional milk would be preferable to cod liver oil *(106)*.

Cod liver oil and sunlight were highly valued for the prevention of rickets, and nurses taught mothers of infants how to use these remedies for their infants *(107)*. From the 1920s to the 1940s, many children in the United States were given cod liver oil each day *(26,65)* with orange juice (which was know to prevent scurvy). However, older

preparations of cod liver oil had an unpleasant taste, the quality of different preparations was erratic *(119)*, and medical professionals became concerned about lipoid aspiration pneumonia *(87)* and vitamin A toxicity *(88)*. By the 1950s, cod liver oil had been largely replaced by synthetic vitamins in the United States; however, the latter do not contain ω-3 fatty acids, which have anti-inflammatory properties *(26)* and important effects on immune function *(47,120)*. In Norway, the Norwegian Nutrition Council continues to recommend supplementation with cod liver oil beginning at age 4 wk, because it provides ω-3 fatty acids in addition to vitamin D *(121)*.

5. THE MODERN ERA

5.1. Over-Fishing of the Oceans

Before Columbus made his first voyage to America, Basque fisherman were secretly fishing the massive stocks of cod and other groundfish off the New England coast *(122,123)*. Their salt cod was a staple in Mediterranean markets, and cod was a staple of the European diet for more than 400 yr *(122)*. Although fishermen exploited cod for centuries, the technological innovations of the 20th century led to the collapse of cod stocks in North America. Motorized boats dragged the ocean floor with massive trawl nets, destroying both cod fish and their habitat. Factory ships with refrigeration have almost erased the limit to the amount of cod that can be caught and sold internationally without spoiling *(123)*; increasingly powerful and accurate sonar produces detailed readouts of nooks where schools of fish may lurk; and shipping fleets can position themselves precisely through use of the satellites of the Global Positioning System *(124)*.

Despite growing regulations on allowable catches and fishing equipment, cod stocks have continued to decrease across the North Atlantic. In 1992, the Canadian government declared a temporary moratorium on cod fishing; the moratorium was extended in 1994. In 2003, with cod stocks showing no sign of recovery, the Canadian government banned all cod fishing off its Eastern provinces and identified some cod populations as endangered. The US government also imposed restrictions on cod fishing *(123)*. However, it is unclear whether North Atlantic cod stocks will recover.

5.2. Pollutants in the Ocean

The level of polychlorinated biphenyls (PCBs) and dioxins in fish and fish oils has become a concern as oceans have become progressively contaminated with industrial waste. This issue was addressed in the United Kingdom and Europe by purity standards *(125)*, which were revised and made more strict in 2002 *(126)*. In the same year, the UK Food Standards Agency reported that exposure to dioxins had decreased by 75% over the previous 20 yr and that the levels of dioxins and PCBs found in most of the samples in their most recent fish oil survey were lower than in previous surveys that were performed in 1994 and 1996 *(126)*. Mercury contamination of fish is also a concern, and the FDA advises that young children and women of childbearing age should avoid tilefish, swordfish, shark, and king mackerel because of their elevated levels of mercury *(127)*. However, an analysis of US fish oil supplements revealed no detectable mercury, with a limit of detection of 0.1 μg of mercury per gram *(128)*.

5.3. Modern Clinical Studies of Vitamin A Supplementation

After a 40-yr hiatus, interest in the anti-infective properties of vitamin A was rekindled in the 1980s by the observation of increased mortality in Indonesian children who

had vitamin A deficiency and xerophthalmia *(65,129)*. The first symptom of eye disease from vitamin A deficiency is night blindness; at this stage, Bitot's spots (superficial, foamy gray, triangular spots) may be present on the conjunctiva *(129,130)*. This is followed in later stages by xerophthalmia (dryness of the conjunctiva), keratomalacia (corneal ulceration), and blindness *(77)*.

Since the 1980s, numerous studies have been performed regarding the effect of vitamin A supplementation on the health of children in developing countries. For a complete review of this subject, the reader is referred to Chapter 23, as well as reviews *(110,131,132)*, and meta-analyses *(67,133,134)*. For the purpose of this chapter, the findings are summarized to provide a basis of comparison to the status of vitamin A in the developed world as well as to provide a perspective on the results of our research.

Vitamin A supplementation of children in developing countries decreased overall childhood mortality by about 30% *(67,132)*. Community-based studies of vitamin A supplementation have indicated that it may decrease the severity, but not the incidence, of diarrhea *(131)*. In children hospitalized with measles in the developing world, vitamin A supplementation decreased mortality by an average of 60% *(67,132,135)*; the decrease in mortality from measles-related pneumonia was particularly noteable *(67)*. The modern studies are consistent with the results of Ellison's historical study of cod liver oil for children who are hospitalized with measles (*see* Section 4.6.). The role of vitamin A supplementation in measles is also consistent with the fact that infectious diseases that induce the acute-phase response transiently depress serum retinol concentrations, that vitamin A deficiency impedes the normal regeneration of mucosal barriers damaged by infection, and that it also diminishes the immune function of white blood cells *(136,137)*.

However, several placebo-controlled trials have demonstrated that high-dose vitamin A supplementation is not effective in decreasing the severity of pneumonia in hospitalized children in developing countries and that large doses of vitamin A may be harmful when given to well-nourished children in these areas *(132,134)*. Additionally, vitamin A supplementation is not effective for children who are hospitalized with pneumonia caused by respiratory syncytial virus, which is a paramyxovirus similar to measles and an important cause of infantile bronchiolitis and pneumonia *(138,139)*. In a multicenter study performed in the United States, patients who received vitamin A actually had longer hospital stays than those who received placebo *(138)*.

Infection with HIV has become increasingly prevalent in many developing countries. Vitamin A supplementation of children younger than age 5 yr who are HIV-positive decreases AIDS-related deaths as well as total mortality and morbidity from diarrhea *(140)*. Small, frequent doses of vitamin A may be more protective than large, periodic doses. Additionally, adequate dietary vitamin A intake is associated with a significant decrease in mortality *(141)*, diarrheal and respiratory infections *(142)*, and stunting *(143)*. New strategies in vitamin A supplementation in developing countries include targeting at-risk populations, improving dietary sources of vitamin A, using horticultural approaches, fortifying food, and addressing multinutrient deficiencies *(140)*.

5.4. Nonclassical Functions of Vitamin D

Modern studies of vitamin D indicate that calcitriol (1,25 dihydroxyvitamin D), the active form of vitamin D, has important nonclassical effects beyond the regulation of calcium metabolism. These include the modulation of hormone and cytokine production and secretion as well as the regulation of proliferation and differentiation *(144)*. Calcitriol, a potent inhibitor of human T-lymphocyte proliferation *(145,146)*, and vitamin D analogs

have been shown to be effective in the prevention and treatment of some models of autoimmune disease in rodents—particularly autoimmune diabetes in mice *(144,147,148)*. In 1997, Muhe and colleagues *(110,149)* reported the importance of nutritional rickets in the development of pneumonia in developing countries. This is consistent with the work of historical authors discussed earlier, who were also aware of this association.

5.5. Discrepancies in Vitamin A Status in the Developed World

Vitamin A deficiency that is severe enough to cause blindness is uncommon in the developed world *(62)*. However, some segments of the US population, particularly socioeconomically disadvantaged children *(93)* as well as African- and Mexican-American children *(62)*, may have suboptimal levels of vitamin A. In 1932, Ellison recognized that children from low-income households were the most likely to have vitamin deficiencies (*see* Section 4.6.) *(108)*. Consistent with these reports, in our original study, five of six children with suboptimal levels of vitamin A were Hispanic general-service patients *(57)*. Additionally, young children in the United States—particularly those in the toddler and preschool age groups—may not have adequate dietary intakes of vitamin A *(150)*.

In developed countries, high intakes of vitamin A (but not β-carotene) by pregnant women have been associated with teratogenesis *(151)*, leading to recommendations that prenatal vitamins should contain no more than 8000 IU of preformed vitamin A *(152)*. Additionally, high intakes of vitamin A by postmenopausal women in the United States *(153)* and 49- to 51-yr-old men in Sweden *(154)* have been associated with a higher risk of hip fractures. As a result, vitamin A supplementation and fortification of food with vitamin A in Western countries has been questioned *(77)*. As discussed under Section 3.3., the amount of vitamin A in Norwegian cod liver oil has been reduced. Nonetheless, the Norwegian Nutrition Council continues to recommend supplementation with cod liver oil beginning at age 4 wk, because it provides ω-3 fatty acids in addition to vitamin D *(121)*.

5.6. Deficiencies of Multiple Micronutrients

Numerous investigators have stated that vitamin A deficiency rarely exists alone and that it is usually accompanied by variety of other nutritional deficiencies *(92,131,132,134,155–158)*. In a 1986 review, Mejía *(92)* noted that vitamin A deficiency primarily affects the world's most underprivileged populations, which, because of their limited socioeconomic condition, also lack a variety of other essential nutrients. He emphasized the importance of the interaction between nutrients and reviewed the established relationships of vitamin A status to protein, dietary fat, vitamin E, zinc, and iron *(92)*. Mejía also mentioned the more controversial links of vitamin A to iodine metabolism; vitamins C, K, and D; calcium, and copper. Realizing that the relationships might be direct or indirect, he emphasized the importance of considering these interactions when "treating or preventing vitamin A deficiency both at the clinical and at the population levels" (ref. *92*, p. 95). Olson *(155)* reported that deficiencies of various other nutrients, including protein, α-tocopherol (vitamin E), iron, and zinc, adversely affects the transportation, storage, and utilization of vitamin A. He also noted that the absorption of vitamin A and carotenoids is markedly reduced when diets contain very little fat (<5 g/d).

More recently, Villamor and Fawzi *(132)* stated that supplementation with vitamins and minerals in addition to vitamin A is likely to "reduce the burden of adverse health outcomes," because of the physiological interactions between nutrients and overlapping

micronutrient deficiencies, including iron and zinc. Semba *(98)* noted that antenatal supplementation with multivitamins reduced fetal deaths and low birthweight in pregnant women who were infected with HIV, but vitamin A alone had no significant effect. Semba *(110)* also discussed the role that other deficiencies of vitamin D *(149)* and zinc *(159)* may have in susceptibility to respiratory infections. We agree with Semba, who stated that "further studies are needed to address the use of vitamin A in multi-micronutrient supplements, as there is increasing evidence that other coexisting micronutrient deficiencies may limit the efficacy of vitamin A" (ref. *131*, p. 105).

6. SUMMARY AND RECOMMENDATIONS

Our work is consistent with the historical uses of cod liver oil, vitamin A as the "anti-infective" vitamin, the link between rickets and respiratory tract infections, the modern understanding of immunomodulatory effects of vitamin D, the importance of ω-3 fatty acids and trace metals in decreasing inflammation, the clinical observation that inflamed respiratory mucosa may not completely recover between episodes of infection, and the current concept of the importance of multiple micronutrient deficiencies.

We have demonstrated that use of flavored cod liver oil (which meets European purity standards) and a chewable children's multivitamin-mineral with trace metals, including Se, can decrease morbidity from upper respiratory tract illnesses, OM, and sinusitis in young children living in the United States. These supplements were particularly well-accepted by Latino families from the Caribbean, where use of cod liver oil is a cultural tradition. Currently, there is adequate information for practitioners to recommend the use of these supplements, when indicated, to their individual patients; information for practitioners and families is available online at http://www.drlinday.com. The supplements can be purchased in the United States without a prescription. Further research is needed to evaluate the effect of the supplements on antibiotic prescription for these illnesses and to explore their role as adjunctive therapy in asthma. Additionally, our findings need to be confirmed in larger studies to facilitate large-scale, policy decision making.

Use of these supplements has the potential to improve children's health and decrease the cost of their health care. However, cod liver oil does not have a National Drug Code number and is not available through Medicaid in New York, and the children's vitamins we have located that are available through this system do not contain Se or other trace metals. Also, cod liver oil is not available through the USDA WIC Program; our request for such availability can be found online at http://www.fns.usda.gov/wic/anprmcomments/ihp-06.pdf. Furthermore, purchase of vitamins with US food stamps is not permitted (*see* http://www.fns.usda.gov/fsp/faqs.htm#9).

Socioeconomically disadvantaged children living in the United States are at risk for micronutrient deficiencies. Although the supplements used in our research can be purchased in the United States without a prescription, their cost may pose an excessive financial burden to low-income families. If our results are confirmed in larger studies, a system change will be needed to provide these supplements to nutritionally vulnerable, socioeconomically disadvantaged children living in the United States.

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22 Micronutrients and Immunity in Older People

John D. Bogden and Donald B. Louria

KEY POINTS

- Placebo-controlled clinical trials, despite their limitations, are the best approach for studying effects of micronutrients on immunity.
- High doses of some single nutrients may improve immunity in relatively short time periods (weeks to months), but persistence of these effects is not currently known. High doses of other micronutrients may adversely affect immunity.
- Some micronutrients may interfere with the beneficial effects of other micronutrients on immunity; this effect depends on relative doses.
- Low- to moderate-dose multivitamin/mineral supplements may require considerable time (6 mo to 1 yr or more) before they enhance immune functions and potentially reduce susceptibility to infectious diseases, and the timing of their effects may differ between men and women.
- High- and even low-dose micronutrient supplements may enhance immunity even in the absence of evidence of underlying deficiencies.
- Micronutrient supplements are not a substitute for a good diet and regular exercise but, rather, are a complementary measure.
- Long-term ingestion of single nutrient supplements, especially at high doses, may have beneficial and/or adverse effects on immunity and other outcomes.

1. INTRODUCTION

Aging has been described as a group of processes that promotes vulnerability to challenges, thereby increasing the likelihood of death. Because there is evidence that depressed immunity can increase the risk of death, it is likely that changes in immunity with age are key factors in the aging process *(1)*.

Theories of aging include the free radical, programmed senescence, and immunological theories *(1,2)*. Evidence for the immunological theory of aging is based largely on the well-described changes that occur with age in various species that have been studied, including man, and on observations from cross-sectional studies that demonstrate an

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association between maintenance of good immune function and longevity *(1,2)*. A limitation of this theory is that it lacks the universality of other theories, such as the free radical theory of aging, because it is not applicable to lower organisms that do not have well-developed immune systems. Of course, the complexity of aging may require the use of more than one theory to understand it, and the various theories are not necessarily independent of one another. For example, there is evidence that demonstrates that antioxidant nutrients that reduce free-radical damage can improve immunity in older people *(3)*, suggesting that the free radical and immunological theories may be complementary.

2. AGING AND IMMUNITY

2.1. General Changes in Immunity With Aging

It is useful to distinguish between primary changes that develop as a result of the age-dependent intrinsic decline of immunity and secondary changes that are the result of "environmental" factors such as prescription and nonprescription drug use, physical activity, and diet. In fact, Lesourd and Mazari *(4)* suggested that secondary, rather than primary, changes in immunity with age are more likely to explain the increased incidence and severity of infectious diseases in older people.

Changes in immunity with aging include inhibited T-lymphocyte functions, decreased antibody production and responses, increased autoimmune activity with compromised self- and nonself-discrimination, and greater heterogeneity in immunological responses *(5–9)*. Regarding the latter, depressed T-cell function is the most common and may begin as early as the sixth decade. However, T-cell dysfunction is neither inevitable nor predictable. For example, we *(8)* measured delayed hypersensitivity skin test responses in 100 people ages 60 to 89 yr. We found that although 41% were anergic to a panel of seven skin-test antigens and an additional 29% were "relatively anergic" (responding to only one of the seven antigens), the remaining 30% were reactive, responding to two or more of the skin-test antigens, often with sizable reactions.

2.2. Specific Changes in Immunity With Aging

2.2.1. Involution of the Thymus

The most striking changes in immunity with increasing age are inhibited T-cell functions (Table 1). These are likely related to the well-known involution of the thymus *(10)*. The differentiation process by which stem cells become T lymphocytes occurs in the thymus. It is a two-lobed structure in mammals, located in the thorax above the heart.

There are several stages in the process by which immature stem cells (pre-T cells) become mature T cells. These include migration to the thymus, where some cells are stimulated to grow and others die; differentiation, in which the mature phenotype of T cells develops in the thymus, including surface expression of accessory molecules; positive selection, in which self-major histocompatibility complex (MHC)-restricted T cells are selected and other cells rejected; and negative selection, which ensures that surviving mature T cells are self-tolerant. The selective survival or death of cells results in a self-MHC-restricted, self-antigen-tolerant, mature T-cell population *(10)*.

The thymus is the principal site of T-cell maturation. Involution with age occurs soon after puberty. Because some maturation of T cells continues throughout adult life, it is likely that a remnant of the thymus or some other tissue continues to effect T-cell

Table 1
Some Specific Changes in Immunity With Aging

Involution of the thymus
Decreased thymic hormone concentrations
Decreased DHST responses
Decreased IL-2 secretion
Decreased LPRs to mitogens
Lower antibody titers after vaccination
Increased serum autoantibodies
Increased soluble IL-2Rs
Reduced phagocytosis by polymorphonuclear leukocytes
Reduced intracellular killing by polymorphonuclear leukocytes

DHST, delayed hypersensitivity skin test; IL, interleukin; LPR, lymphocyte proliferative response; IL-2R, interleukin-2 receptor.

maturation *(10)*. However, because memory T cells have a long life span (20 yr or more) *(10)*, the involution of the thymus does not cause compromised immunity in young adults but is likely to contribute to depressed immunity as the time since thymic involution increases.

The involution of the thymus prior to the peak reproductive years suggests that this process may provide an evolutionary advantage. One hypothesis is that involution provides a net benefit, because it reduces the risk of autoimmune reactions *(11)*. According to this theory, the increased risk of cancer or infectious diseases resulting from depressed cellular immunity is a detriment that is offset by a reduced risk of autoimmune disease that accompanies thymic involution. Although attractive, this theory of immunological "trade-offs" as an adaptation to aging requires additional supporting evidence.

An alternative hypothesis was proposed by Siskind *(12)*, who suggested that adaptation to environmental pathogens occurs early in life and, thereafter, relative constancy of immune function rather than adaptability may be most beneficial. He further speculated that efforts to modify cellular immunity in later life (e.g., by pharmacological or nutritional means) possibly does more harm than good. Although interesting, this hypothesis is not widely supported and not consistent with the known association between good cellular immunity and reduced morbidity and mortality in older individuals.

2.2.2. T-Lymphocyte Functions

Changes in T lymphocytes with aging include a shift in relative percentages of subpopulations and qualitative changes in cell surface receptors *(13)*. In comparison to T cells from younger people, cells of the elderly are deficient in in vitro production of certain T-cell growth factors such as interleukin (IL)-2 and have a decreased ability to bind and respond to it *(14–17)*. McMurray *(15)* outlined evidence that implicated nutrient-mediated effects at virtually every step in the development and expression of T-cell immunity, from direct effects on the thymus and thymic hormone production through T-cell maturation and distribution, antigen reactivity, lymphokine production, and even composition of the T-cell membrane.

Delayed hypersensitivity skin test (DHST) responses involve T-lymphocyte proliferation, production of IL-2 and other lymphokines, and infiltration of the test site with mononuclear cells, resulting in induration and erythema 24 to 72 h later; it is the T-cell parameter that is most consistently and profoundly affected by nutritional status *(15)*. Reduced DHST responses are also the immune parameter most consistently associated

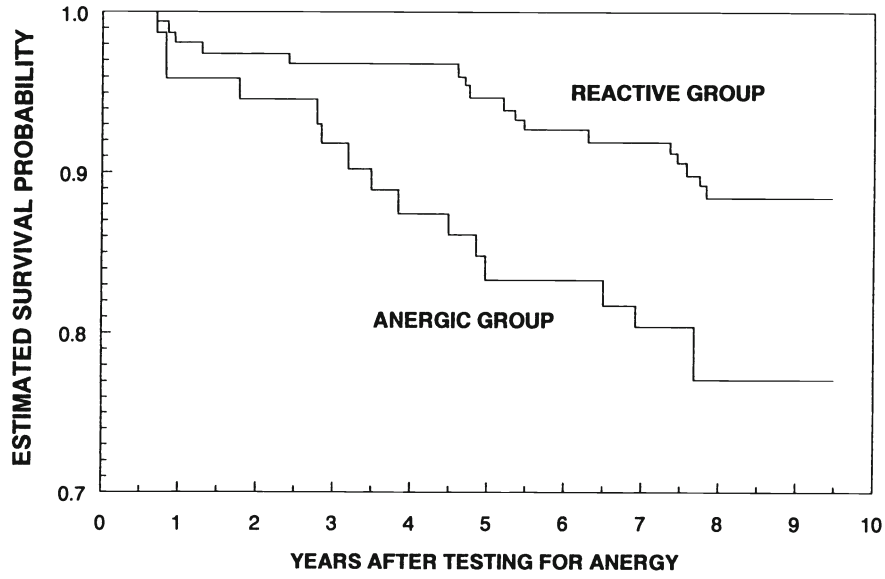

Fig. 1. Kaplan-Meier curves of all-cause mortality for initially anergic and reactive older people during 10 yr follow-up period. Subjects (n = 273) were aged 60 or older and apparently healthy at enrollment. Participants were considered anergic if their response to each of four skin test antigens were less than 5 mm of induration. Most of the excess mortality in the anergic group occurred within 5 yr of enrollment. (Adapted from Wayne et al. *[20]).*

with increased infectious disease morbidity and mortality from all causes in older people, as discovered by Meakins et al. *(18)*; and Christou et al. *(19)* in surgery patients, and Wayne et al. *(20)* and Roberts-Thomson et al. *(21)* for initially healthy people ages 60 yr or older.

In their investigation, Christou et al. *(19)* studied the relationship between presurgical DHST responses and postsurgical sepsis-related death in 245 subjects with a median age of 67 yr and a range in age of 24 to 98 yr. Initially, anergic subjects experienced significantly more postsurgical mortality than those who were reactive. Because all the subjects had gastrointestinal cancers that prompted the decision to operate, it could be argued that the initial severity of the disease increased both the incidence of anergy and the risk of dying postoperatively. Thus, initial disease severity could explain the apparent strong relationship between preoperative DHST responses and postsurgical mortality. However, the study by Wayne et al. *(20)* did not possess this confounder, because they looked prospectively at healthy adults over a 10-yr period. In this investigation, the authors followed 273 initially healthy subjects ages 60 yr or older who had no history of serious medical problems. DHST responses to four recall antigens were measured at enrollment. Anergy (failure to respond to any skin-test antigen) at enrollment in the study was associated with a significantly increased risk of dying in the 10-yr follow-up period. For example, at the end of 10 yr, 89% of the initially reactive subjects were still alive, but 22% of the anergic subjects had died. The study demonstrates that anergy to skin-test antigens, even when present in healthy older people, is associated with subsequent increased all-cause mortality. The authors also found a 2.5-fold increase in cancer mortality in the initially anergic group in comparison to the reactive group. However, this was not statistically significant, probably because of the relatively small number of cancer deaths observed.

Evidence for the decline in T-cell function with age includes a considerable number of studies that demonstrate reduced lymphocyte proliferative responses (LPRs) to mitogens or antigens, as well as depressed DHST responses to recall antigens *(7,16–23)*. Indeed, these two measures of T-cell function have been the most widely studied functional tests performed in conjunction with assessment of the effects of nutritional intervention on immunity. A problem with LPRs to mitogens is the considerable variability of these assays, even in laboratories with rigid quality-control procedures.

There is some evidence for changes in T-lymphocyte subsets with aging—particularly decreases in $CD4^+$, increases in $CD8^+$ cells, and decreases in the $CD4^+/CD8^+$ ratio *(9)*. There also is evidence that lymphocyte subsets are altered in elderly people who are ill. For example, Markewitz et al. *(24)* found that immunosuppression in cardiopulmonary bypass surgery patients ages 55 yr or older was associated with decreased $CD4^+$ T cells and increases in $CD8^+$ T cells. Higa et al. *(25)* found that increases in $CD8^+$ T cells predicted a longer period of recovery after onset of acute herpetic pain during herpes zoster infection. The increased incidence of this disease in older people is believed to result from the depressed cellular immunity that occurs with age *(14)*.

Measurement of lymphocyte subsets is a key component in evaluation of immune function *(26,27)*. Knowledge of lymphocyte subset numbers (cells/milliliter and percent of total) allows determination of relationships between immune functions and the number and percentage of cells responsible for these functions. This can permit one to distinguish between effects caused by increased numbers of a particular subgroup of cells vs enhanced activity by the same number of cells. The latter could be related to antigen-binding capacity per cell. Indeed, changes in antigen-binding capacity per cell could be a mechanism by which micronutrients influence immune functions. However, the importance of changes in antigen-binding capacity per cell in declining immunity with age is largely unexplored.

2.2.3. Other Immune System Changes With Aging

There is some evidence for a decline in B-cell functions with age, although it is likely related, at least in part, to the T-cell dependence of B-cell functions. Older people vaccinated with tetanus toxoid, varicella-zoster, or hepatitis B antigens demonstrate reduced antibody production as well as a greater percentage of nonresponders compared to young adults. This may also be true after pneumococcal and influenza virus immunization, although the evidence is not as convincing *(28)*.

Perskin and Cronstein *(29)* reported that aging produces alterations in neutrophil plasma membrane viscosity that may result in compromised neutrophil function and increased susceptibility to infection with specific pyogenic bacteria. This is consistent with studies of Nagel et al. *(30)*, Shoham-Kessary and Gershon *(31)*, and Corberand et al. *(32)* that suggest compromised in vitro activity of neutrophils from older people. Depending on the microorganism studied, the neutrophil activity that was depressed was phagocytosis or intracellular killing.

A review by Makinodan et al. *(33)* suggested that although antigen-responsive cells such as B cells, monocytes, and natural killer cells are vulnerable to aging, T cells are clearly the most vulnerable. Therefore, most studies regarding nutrition, immunity, and aging have focused on T-cell functions.

Sen et al. *(28)* published an insightful review that distinguished between an increased incidence vs greater severity of infectious diseases in elderly people. For example, they reported an increased case-fatality ratio for bacterial meningitis and pneumococcal pneumonia in older people as well as an increased incidence of diseases such as urinary

tract infections and varicella zoster. Other diseases such as influenza virus infection and Gram-negative sepsis are both more frequent and more severe in older people. They suggested that in addition to changes in immunity with age, local urinary tract, respiratory tract, and neurological changes possibly contribute to the increase in infectious disease morbidity and mortality in older people.

Relationships among the ILs, their receptors, and immunity have been discussed widely in the recent literature. Of particular interest in the elderly is IL-2, because its production is decreased in older individuals *(9)*. Interestingly, soluble IL-2 receptor (IL-2R) levels are higher in older than in younger adults *(34)*, and it has been suggested that this may be a factor in the decline of cellular immunity with age, because high serum concentrations of soluble IL-2R may compete with and decrease IL-2 binding to T-cell IL-2Rs and thereby compromise immunity *(35,36)*. We found that serum IL-2R concentrations are relatively lower in physically active older people compared with sedentary seniors and that exercise/physical activity habits and multivitamin supplementation may interact to influence soluble serum IL-2R concentrations *(37)*. We also verified the higher levels of soluble IL-2R in older people (unpublished data, 1998).

There may be a "survivor" aspect to the relationship between advanced age and immune capacity. The oldest people, including centenarians studied by Sansoni et al. *(38)*, tend to have well-preserved immune functions, such as natural killer (NK) cell activity, that are often better than those of 50- to 80-yr-olds. In addition, those above age 90 tend to have lower serum auto-antibody concentrations than those in the 60- to 80-yr range *(14,39)*. Thus, enhanced immunity and reduced autoimmunity appear to be associated with the ability to live to age 90 yr and beyond.

3. MICRONUTRIENTS AND IMMUNITY

3.1. Nutrition, Immunity, and Aging

Scrimshaw and San Giovanni *(40)* noted that infections, no matter how mild, can adversely affect nutritional status, which in turn can compromise immunity and exacerbate the effects of infection. They discussed evidence for the effects of various micronutrient deficiencies on immunity, including β-carotene; pyridoxine; folic acid; pantothenate; vitamins A, B_{12}, C, D, and E; the trace elements iron, zinc, and copper; and magnesium. Generally, cell-mediated and nonspecific immune functions are more sensitive to single micronutrient deficiencies than humoral immunity.

Fraker *(41)* noted that the immune system is a large "organ" comprised of the blood, spleen, lymphatic system, thymus, and other components. Additionally, millions of new immune system cells are produced daily. Its large size and high cellular turnover combine to make the immune system a major consumer of nutrients. Therefore, it is not surprising that some aspects of immunity are very sensitive to nutritional deficiencies.

One key issue is whether the decline in immunity with aging results, at least in part, from nutritional deficiencies and/or increased requirements. Another possibility is that micronutrient supplementation might improve immunity even in the absence of an underlying "deficiency," defined by factors such as low circulating nutrient concentrations or consistently low intakes.

Human studies of protein-calorie malnutrition in underdeveloped countries or in hospitalized adults demonstrate a causal association between undernutrition and secondary

immunodepression that results in diminished resistance to infectious diseases *(14,15,42,43)*. This association is consistent enough to permit the use of DHST responders as a predictor of clinical prognosis in medical and surgical patients *(19)*. Thus, there appears to be little doubt that severe malnutrition has a major impact on resistance to disease that is partly mediated through the immune system. There is also evidence that moderate-to-marginal undernutrition may compromise immunity *(44,45)*.

McMurray *(15)* noted that dietary deficiencies, both moderate and severe, of specific nutrients profoundly altered cell-mediated immune responses in humans and experimental animals. Diets with inadequate contents of calories, protein, vitamin A, pyridoxine, biotin, or zinc can result in depressed production of thymic hormones critical for T-lymphocyte differentiation. Reduced numbers and depressed in vitro function of T cells also have been reported in experimental deficiencies of zinc, copper, iron, and vitamins A and E. Depressed DHST responses are a consistent result of dietary inadequacies of protein, pyridoxine, iron, zinc, and vitamins A and C.

The classic review by Beisel *(46)* extensively examined the literature up to 1982 on single nutrients and immunity. The water-soluble vitamins that appear to be most critical for maintaining immunity are vitamin B_6, folate, vitamin B_{12}, and vitamin C. Among the lipid-soluble micronutrients, vitamins A and E appear to exert the most significant impacts. Recent studies have shown that vitamin D is also an important immune modulator. Trace metals that exert substantial influences on immune functions are iron, zinc, selenium, and copper *(15,46)*.

Because the variability in immune responses increases with aging, subgroups that have impaired immunity because of nutrient deficiencies are more likely to be observed in the elderly than in other age groups. Additionally, when episodes of nutritional vulnerability overlap with suboptimal immune function, an adverse synergistic interaction is possible *(15)*. These factors make it more rewarding to study nutrition–immunity relationships in older rather than younger adults. Beisel *(47)* noted that individual studies of immunity in humans were not systematic or comprehensive. This is no doubt due to the very considerable expense that would be incurred in studying multiple immune responses in a sizeable number of older people.

It can be useful to compare relationships between nutrition and immunity in older people with those of people with diseases in which immune functions are compromised. In the case of HIV infection, we *(48)* found that compromised nutritional and antioxidant status began early in the course of infection and possibly contributed to disease progression. This observation can be compared to the decline in cellular immunity that begins in many older people in the fifth or sixth decade and eventually is associated with a reduced life span *(2,5,20)*.

3.2. Cross-Sectional Studies on Micronutrient Nutrition and Immunity

Goodwin and Garry *(49)* compared immunological functions of healthy elderly New Mexico residents consuming higher than Recommended Daily Allowance (RDA) levels (five times the RDA or greater) of micronutrients with similar individuals not taking supplements. Micronutrients evaluated were vitamins A, C, D, and E, the B vitamins, iron, calcium, and zinc. There was no significant difference between the two groups in DHST responses or in vitro LPRs to mitogens. The authors suggested that the immune-enhancing properties of high doses of vitamins might be the result of a nonspecific adjuvant effect that does not persist with time.

More recently, the same authors *(50)* studied 230 healthy older men and women to determine if subclinical micronutrient deficiencies could contribute to the depressed immunity found in many of the elderly. Immune functions studied included DHST responses, in vitro LPRs to phytohemagglutin (PHA), lymphocyte counts, and levels of serum auto-antibodies. Spearman correlation coefficients were calculated to assess associations between blood micronutrient concentrations and selected immune functions. The authors also compared subjects with the lowest responses to those with the highest. There were no significant associations between low serum micronutrient concentrations and immune functions, and the authors suggested that subtle nutrient differences did not appear to contribute to the immunodeficiency of aging. However, the population sample studied was relatively affluent; people taking prescription drugs or daily over-the-counter medications, as well as those with a serious medical problem, were excluded. Therefore, the study may have excluded those subjects who might benefit most from micronutrient supplements. Kawakami et al. *(51)* studied 155 healthy subjects ages 20–99 yr and suggested that cell-mediated immunity was reduced as a result of malnutrition.

In a more recent study, *(52)* we examined relationships between immunity and dietary and serum antioxidants, B vitamins, essential trace metals, and serum homocysteine in 65 older men and women ages 53 to 86 yr. Subjects who had used vitamin or mineral supplements in the preceding 3 mo were excluded. Soluble serum IL-2R concentrations (sIL-2R) were positively associated with body mass index and serum concentrations of homocysteine and vitamin B_6 and negatively associated with serum β-carotene and dietary lycopene. In a multiple regression model, these five factors explained 52% of the variability in sIL-2R. A total of 25% of subjects displayed anergy to a panel of seven recall skin antigens, and these responses were negatively associated with T-helper cell number, suggesting the reduced numbers of the latter was a possible factor that may have contributed to the anergy of the subjects. T-helper cell numbers were positively associated with serum copper, and NK cell numbers were positively associated with dietary folate and vitamin B_6. The results document relationships between nutrition and immunity and suggest that IL-2 may be influenced by dietary antioxidants and B vitamins, including those that modify homocysteine metabolism.

The studies mentioned earlier were not attempts to intervene by provision of micronutrient supplements; rather, they were assessments of associations between the subjects' usual intakes or blood concentrations and selected immune functions. Variables that cannot be controlled in cross-sectional studies may mask associations between nutritional factors and immunity, especially because immunity is likely to be dependent on a number of factors, only one of which is nutritional status. Such studies are valuable as a way to identify nutrients for more intensive study but can only provide statistical associations that may not be cause–effect relationships. The latter can be assessed by standard placebo-controlled, double-blind clinical trials.

3.3. Clinical Trials of Single Micronutrients

Several clinical trials have been conducted in recent years. These have included depletion—repletion studies in young volunteers and provision of micronutrient supplements to older people who did not appear to have pre-existing deficiencies.

Jacob et al. *(53)* studied the effects of short-term ascorbate depletion on immunity and other factors in young adult males confined to a metabolic ward. Ascorbate depletion was achieved using daily doses of 5 to 20 mg/d, whereas repletion was achieved with doses of

60 to 250 mg/d (60 mg was the RDA at that time). Although LPRs to mitogens were not affected by ascorbate depletion–repletion, delayed DHST responses to a panel of seven recall antigens were markedly depressed by ascorbate depletion. Repletion for 28 d at either 60 or 250 mg/d did not restore the mean antigen score to the predepletion level, although there was some improvement in induration in three of the eight men studied. These results suggest that DHST responses are more sensitive to ascorbate depletion than mitogen responses. They further suggest that the repletion period was of insufficient duration to produce a return of DHST to baseline levels and/or the repletion doses were not large enough.

Fuller et al. *(54)* studied the effect of β-carotene supplementation on the ultraviolet (UV) radiation-induced photosuppression of DHST responses in 24 young adult males, ages 19 to 39 yr. They found that exposure to a UV A/B light source over a 16-d period significantly reduced DHST responses in a control (placebo) group to 39% of the initial values, but did not induce significant reductions in a group given 30 mg of β-carotene per day. Because young men were studied, the question of whether these results would occur in young women or in older men and women was not known. This group then repeated the study in elderly men and found similar effects of UV suppression that was prevented with β-carotene supplementation, although there was more variability in DHST responses in the older subjects compared to young adults *(55,56)*.

Watson et al. *(57)* investigated the effects of β-carotene on lymphocyte subpopulations in male and female subjects with a mean age of 56 yr. β-carotene was given at doses of 15, 30, 45, or 60 mg/d for 2 mo. Using monoclonal antibodies to identify lymphocyte subsets, they found that the percentages of T-helper and NK cells, as well as cells with IL-2 and transferrin receptors, were increased in a dose-related fashion. There were no significant effects of β-carotene on T-suppressor cells. However, the number of subjects in each treatment group was only three to five; thus, further investigation is needed to confirm these findings.

Santos et al. *(58)* found that men participating in the Physicians' Health Study who consumed 50 mg of β-carotene on alternate days for an average of 12 yr had significantly greater NK cell activity than controls given placebos. Surveillance by NK cells is considered to protect against the development of cancer. However, two large intervention trials found an association between high doses of β-carotene and the development of lung cancer in cigarette smokers *(59,60)*. The role of the immune system in leading to the development of lung cancer in these studies is not known, but the results suggest that there are risks associated with the long-term use of high doses of β-carotene supplements in smokers.

In a pilot study, Talbott et al. *(61)* investigated the impact of pyridoxine supplementation on lymphocyte responses in 15 older (ages 65–81 yr), mostly female subjects and found that administration of 50 mg/d of pyridoxine hydrochloride significantly increased in vitro LPRs to PHA, pokewood mitogen, and *Staphylococcus aureus*.

Meydani et al. *(62)* reported that vitamin B_6 deficiency impaired IL-2 production and lymphocyte proliferation in older adults. Each of these measurements was reduced by about 50% by depletion, whereas repletion with near-RDA levels of B_6 eventually increased values to about the baseline levels. Although only eight subjects were studied, this well-designed investigation supports a number of other studies that suggest that vitamin B_6 may play a key role in immune responses *(63)*.

In another study, Meydani et al. *(64)* gave older people 50, 200, or 800 mg of vitamin E daily for 4 to 5 mo. This resulted in improved antibody titers to hepatitis B vaccine and enhanced DHST responses, especially in the group consuming 200 mg of

vitamin E per day. This suggests 200 mg as a recommended dose, although lower doses may be equally effective when administered for longer periods of time. In a more recent study, Pallast et al. *(65)* investigated the effects of supplementation of healthy older men (ages 65–80 yr) with vitamin E for 6 mo at doses of 50 and 100 mg daily. There was a dose-related trend of increased DHST responses, especially in those subjects with initially low responses, suggesting that there are subgroups of older people that might benefit most from vitamin E supplements.

There has been considerable interest in the potential for zinc to improve immune functions in older people. It is clear that severe zinc deficiency in animals and people (e.g., as found in the disease *acrodermatitis enteropathica*) can greatly compromise cellular immunity and lead to the development of life-threatening opportunistic infections *(66)*. There are also reports of significant associations between plasma or cellular zinc concentrations and immune functions such as DHST responses in older people *(8,67)*. However, recent studies of the impact of zinc supplementation on immunity in older people have not been encouraging. They have demonstrated either no beneficial effect of zinc supplements on immunity or an adverse effect even when the supplements contained modest doses of zinc in the range of 15 to 25 mg/d *(68,69)*. In the absence of an underlying deficiency, use of zinc supplements by older subjects, especially at doses that exceed 15 mg/d, is more likely to adversely affect immunity than improve it. The effects of zinc on immunity in the elderly were recently reviewed *(70)*.

Doherty et al. *(71)* studied the effect of low (1.5 mg/kg) vs higher (6.0 mg/kg) zinc supplementation on mortality in 141 young children in Bangladesh with protein-energy malnutrition, and weight-for-age z scores of about −4.6. Mortality was significantly greater in the high-dose group, with sepsis a frequent contributing factor. The results suggest that high-dose zinc supplementation may contribute to increased risk of sepsis and mortality in severely malnourished children. Although this study involved only very young children (ages 6 mo to 3 yr), it suggests caution in the use of high-dose zinc supplements by any age group.

3.4. Clinical Trials of Combinations of Micronutrients

The studies discussed earlier focused on the effects of relatively large doses of individual micronutrients on immune functions. There have been a limited number of published placebo-controlled trials on the effects of multivitamin/mineral supplements on immune functions in older people.

In the first of these studies, we investigated the effects of zinc given in combination with a multivitamin on immune functions in 63 older subjects *(72)*. All subjects received a standard multivitamin/mineral supplement that contained all of the essential micronutrients, with the exception of zinc. Additionally, subjects received 15 or 100 mg of zinc or a placebo. Daily consumption of the multivitamin/mineral supplement for 1 yr was associated with enhanced DHST and mitogen responses, but these effects were reduced and delayed by ingestion of 15 and, especially, 100 mg of zinc each day. These data suggest that interactions among micronutrients may influence their effects on immunity and that some individual micronutrients—even at modest doses—may have unexpected adverse effects. The adverse impact of zinc is consistent with other previously cited recent studies that indicated that zinc supplements in healthy older individuals either do not improve immunity or adversely affect it *(68,69)*.

Penn et al. *(73)* studied the effects on immune functions of a supplement containing vitamin C (100 mg), vitamin A (8000 IU), and vitamin E (50 mg); it was given for 28 d to half of the 30 elderly subjects studied. All subjects were patients who had been hospitalized for at least 3 mo. The number and percent of $CD4^+$ and $CD8^+$ T cells were significantly increased in the supplemented group but not in a placebo group. Proliferative responses of lymphocytes to the mitogen PHA were also significantly increased in the supplemented group by 64 to 283% but were not affected by the placebo. There was biochemical evidence of deficiencies of vitamins A, C, and/or E in 5 to 47% of the supplemented subjects at enrollment into the study. Therefore, it is possible that the improvement in cellular immunity in these subjects with short-term administration of vitamins A, C, and E was the result of correction of underlying deficiencies that are more likely to be present in hospitalized than in independently living older people. These results suggest that this group of micronutrients may be particularly important for enhancement of immune responses in older people.

In another study, Chavance et al. *(74)* enrolled 218 subjects ages 60 yr or older who were living independently and had not used any vitamin supplements for at least 3 mo prior to the study. They were given a low-dose multivitamin or placebo for 4 mo. No clinical or laboratory assessments of immune function were conducted. The authors found no significant effects of supplementation on the incidence of infections; however, effects on the duration of each infection or the total number of days of infection were not assessed. As suggested by the authors, the failure to find any significant effects on the incidence of infections may have resulted from the short duration of supplementation. This is consistent with our results *(72)*, which suggest that periods of supplementation of about 12 mo are required before improvements in immune functions occur in older people.

We also conducted a randomized, placebo-controlled, double-blind trial of the effects of RDA-level micronutrient supplementation on plasma vitamin and trace metal concentrations and immune functions in independently living healthy older subjects *(75)*. The over-the-counter micronutrient supplement used in the study contained RDA levels of each of the essential vitamins and low-to-moderate doses of minerals.

Of the 65 subjects enrolled, 56 (86%) completed the 1-yr study. About two-thirds were females. As expected, there were no statistically significant effects of the placebo on plasma micronutrient concentrations. In contrast, the data for the micronutrient supplement group show statistically significant increases at 6 and/or 12 mo for plasma concentrations of ascorbate, β-carotene, folate, vitamin B_6, and α-tocopherol. These data verify that supplementation with RDA levels of the latter micronutrients can increase their plasma concentrations in older individuals.

Table 2 contains the data on DHST responses for all study subjects combined and for males and females separately. For induration in the placebo group, there were no statistically significant differences between the 0- and 6-mo results, 0- and 12-mo results, or 6- and 12-mo data. Similar results were obtained for the analyses of the data for the placebo group regarding the number of positive responses.

For the micronutrient supplement group, there was also no significant difference for the data on induration at 0 and 6 mo. However, there was a statistically significant difference between the 0- and 12-mo induration results ($p = 0.005$). There was an increase in induration between 6- and 12-mo, but this did not achieve statistical significance ($p = 0.056$). Similar trends were observed for the individual skin-test antigens.

Table 2
Delayed-Hypersensitivity Skin-Test Responses of Placebo and Micronutrient Groups[a]

Subgroup and response type	*Placebo group*			*Micronutrient group*		
	0 mo	*6 mo*	*12 mo*	*0 mo*	*6 mo*	*12 mo*
All subjects						
Positive responses	1.65 ± 0.30	1.42 ± 0.25	1.73 ± 0.29	1.45 ± 0.25[b]	1.76 ± 0.27[b,c]	2.38 ± 0.33[c]
Total induration, mm	5.37 ± 1.02	4.76 ± 0.93	5.80 ± 0.95	5.21 ± 0.98[b]	5.73 ± 0.94[b,c]	8.40 ± 1.25[c]
Males						
Positive responses	2.93 ± 0.60	1.93 ± 0.30	2.50 ± 0.78	1.64 ± 0.33[b]	2.59 ± 0.43[b,c]	2.86 ± 0.53[c]
Total imduration, mm	8.86 ± 1.91	6.36 ± 1.29	8.88 ± 2.51	6.23 ± 1.15	8.85 ± 1.58	10.91 ± 2.08
Females						
Positive responses	1.18 ± 0.29	1.24 ± 0.31	1.45 ± 0.27	1.33 ± 0.36[b]	1.25 ± 0.29[b,c]	2.08 ± 0.42[c]
Total induration, mm	4.08 ± 1.09	4.17 ± 1.16	4.67 ± 0.83	4.58 ± 1.41[b]	3.83 ± 0.95[b]	6.86 ± 1.49[c]

[a]Mean ± SE; $n = 26$ for placebo group (7 males, 19 females), $n = 29$ for micrantrient group (11 males, 18 females). Positive responses are the mean number of antigens eliciting a response from a total of seven antigens. Total induration is the sum of the indurations of all positive responses. Within groups, values in the same row with different letter superscripts are significantly different, $p < 0.05$ (Wilcoxon signed-rank test).

Similar results also were obtained for the number of positive responses in the micronutrient treatment group. The mean number of positive responses in the placebo group increased by only 4.8% between 0 and 12 mo, and induration increased by 8%. In contrast, in the micronutrient supplement group, the mean number of positive responses increased by 64% and induration by 61% between 0 and 12 mo. These data provide strong evidence for the enhancement of DHST responses after 1 yr of micronutrient supplementation.

The results also suggest that some enhancement of DHST responses occurred sooner (at 6 mo) in the male subjects than in the females (Table 2). The male subjects had significantly greater DHST responses than the females at enrollment; this is consistent with previous data that suggest that DHST responses in males may differ from those in females *(76)*. The diets of the male subjects differed from the females because they were higher in energy intake as well as intake of individual micronutrients, and it is possible that this factor may have interacted with micronutrient supplementation to influence DHST responses.

There was an increase between 0 and 12 mo in the number of subjects in the placebo group with low blood concentrations of some of the micronutrients measured, specifically β-carotene, retinol, folate, and vitamin B_6. This trend differed significantly from the micronutrient group, for which the number of low values changed very little between 0 and 12 mo. Therefore, the improvement in skin-test responses in the micronutrient group did not result from the correction of underlying micronutrient deficiencies for the nine micronutrient concentrations that we determined in blood, at least as defined by current guidelines for low circulating concentrations. The increased number of low values in the placebo group suggests that older people who do not take vitamin supplements for 1 yr may have an increased risk of developing one or more low concentrations—particularly for vitamin B_6, folate, and β-carotene.

Our data suggest that enhancement of immune functions in older subjects by low-dose micronutrient supplementation takes approx 1 yr. These results also suggest that the diets of older people are inadequate in one or more micronutrients and/or that the current RDAs for one or more micronutrients may be too low to support optimal immunity in older adults. For optimal responses, they required the RDA level of the vitamins in the supplement in addition to the amounts in their food.

It could be argued that a 60% increase in DHST responses over a 1-yr period is only a mean increase of about 5% per month. However, this increase far exceeds the decline in DHST responses per year that occurs with aging and, thus, may completely prevent it. These results suggest that older subjects who take a "one-a-day"-type multivitamin supplement faithfully for at least 6 to 12 mo may experience a substantial improvement in measures of cellular immunity such as DHST responses. It is possible that more rapid and/or larger increases in DHST responses would occur if higher doses of micronutrients were used.

More recently, Girodon et al. *(77)* studied the effects of trace element and vitamin supplementation on immunity and infections in institutionalized subjects ages 65 yr and older in France. Daily for 2 yr, subjects (n = 725) received a placebo; a trace-element supplement containing 20 mg of zinc and 100 μg of selenium; a vitamin supplement with 120 mg of vitamin C, 6 mg of β-carotene, and 15 mg of vitamin E; or both the vitamin and trace-element supplements. DHST responses were not significantly influenced by any treatment, but antibody responses to influenza vaccine were improved in the groups given zinc and selenium, and the incidence of respiratory tract infection was

marginally lower ($p = 0.06$) in these groups. The vitamin and trace-element supplements also reduced the prevalence of underlying deficiencies of these nutrients. Because these were institutionalized subjects with a high frequency of low blood micronutrient concentrations, the applicability of these results to healthy independently living people is uncertain. Nevertheless, this large study provides the first evidence that selenium may be a key nutrient in the maintenance of immunity in older people.

3.5. Limitations of Current Knowledge

The previously mentioned studies that focused on the effects of multivitamins on immune functions, in combination with the short-term higher dose single nutrient studies such as those of Meydani *(62,64)*, Watson *(57)*, and Talbott *(61)*, provide solid evidence that micronutrient supplements can enhance immune functions in older people, but data regarding effects on the incidence and prevalence of infectious diseases are quite limited. Despite the evidence provided by these studies, we do not know if long-term daily use of multivitamin/mineral supplements enhances immune functions and reduces the incidence and severity of infectious diseases in older people beyond the 1- to 2-yr duration of the longest studies done to date. This is an unfortunate gap in our knowledge, because millions of older Americans currently consume a multivitamin/mineral supplement daily, either alone or in combination with one or more single nutrients at higher doses *(78,79)*. This situation is partly the result of the limited objectives of all previously completed studies. All of the single nutrient studies have been of short duration, usually using high doses of one micronutrient given to a relatively small number of subjects. Most of these studies have not assessed the impact of single nutrient supplementation on the incidence of infectious diseases—a limitation related to the small number of subjects enrolled in these studies and their short duration, with a consequent lack of statistical power to assess disease incidence. The studies multivitamin and/or trace element supplements also have the following limitations:

1. The study by Chavance et al. *(74)* was only 4 mo in duration. Although, this study assessed the impact of multivitamin supplementation on the incidence of infectious diseases, it did not include any measures of immune function,
2. The study of Penn *(73)* was only 1 mo in duration and included only older people who had been hospitalized for at least 3 mo.
3. Our studies *(71,75)* assessed DHST responses, LPRs to mitogens, and NK cell activity; however, we could not examine other measures of immunity or clinical outcomes, and the period of supplementation was limited to 1 yr.
4. The 2-yr study by Girodon et al. *(77)*, although promising, included only institutionalized subjects.

Thus, additional studies of micronutrient—immunity—disease relationships are required, particularly studies that focus on clinical outcomes.

4. FACTORS THAT CAN INFLUENCE NUTRITION–IMMUNITY RELATIONSHIPS

Factors that may influence micronutrient—immunity relationships in older people include gender, stress, disease, physical activity and exercise, obesity, and food choices.

In our recent study *(75)* of the effects of low-dose micronutrient supplements on immunity in older people, improvements in DHST responses occurred sooner in the

males than the females. Although the reason for this is not known, one possibility is that the higher intake of micronutrients from food in the men results in a larger total micronutrient intake. As mentioned previously, this effect may be a consequence of the generally greater energy and micronutrient intakes of males.

There is a considerable number of reports that psychological and physiological stress in experimental animals and people can depress cellular immune functions *(80,81)*, although it is beyond the scope of this chapter to assess these studies in any detail. As an example, death of a spouse has been associated with depressed immune functions *(81)*. However, virtually all studies of relationships between stress and immunity have not adequately assessed nutritional factors that may be altered by stress and, in most cases, have completely ignored nutrition. Physical and psychological stress can modify food intake in animals and people; thus, studies of stress—immunity relationships usually are confounded by nutritional factors that have not been adequately evaluated.

There is considerable evidence that physical activity/exercise patterns can influence immunity *(82–86)*. Generally, the data suggest that very strenuous exercise can acutely depress immunity. For example, various studies have demonstrated that participants in marathons have a significantly increased risk of respiratory infections in the 1- to 2-wk period following the race *(85,86)*. Chronic overtraining also has been associated with depressed immunity *(84)*. In contrast, regular moderate exercise appears to enhance immune functions *(84)*. One hypothesis is that regular exercise contributes to the maintenance of muscle mass, and muscle is the source of a key nutrient, glutamine, which is required by lymphocytes *(87)*. Additionally, alterations in cytokine levels as a result of regular exercise may be a factor *(88,89)*.

In a review, Nieman *(90)* concluded that infection risk following intensive exercise likely is related to acute nonpersistent changes in immunity. However, unless the athlete exceeds his or her usual training limits, immunocompromise is unlikely, although further research is needed to confirm this conclusion. Generally, studies of macronutrient or micronutrient supplements in combination with exercise have shown no effects on immunity, with the exception of attenuated humoral and cellular immune responses associated with carbohydrate consumption. For example, in a randomized trial of 112 elderly (mean: 79.2 ± 5.9 yr) men and women, Paw et al. *(91)* reported that exercising two times a week improved DHST responses to recall antigens, but consumption of micronutrient-enriched foods (25–100% of the RDA for various micronutrients) did not enhance the effect of exercise.

Stallone *(92)* outlined studies that indicated that excess body weight in humans or experimental animals was associated with impairments in host defense mechanisms. Definitive studies have not been done, but there are data suggesting both beneficial and detrimental effects of weight loss on immunity. In experimental animals, it is well-known that chronically reduced energy intake without malnutrition can profoundly ameliorate the detrimental effects of aging on immunity and can increase mean and maximum life span *(93)*.

The well-established importance of some micronutrients, such as vitamin E, in the maintenance of immune function suggests that choices of foods high in these micronutrients may be beneficial, but this has not been validated in well-controlled studies.

In recognition of the evolutionary development of humans as hunter–gatherers who consumed foods but not supplements, it has been argued that appropriate food choices are sufficient to achieve optimal health, including optimal immunity. A substantial body of

evidence supports the finding that wise food choices, including diets high in fruit and vegetable intake and low in saturated fat, are key factors to the prevention of some chronic diseases. However, arguments based on evolution are compromised by two factors. First, evolution has programmed humans and other species to live through our peak reproductive years but not necessarily beyond them. Second, our preagricultural ancestors had intakes of some nutrients (e.g., calcium, iron, and zinc) much higher than those of modern humans *(94)*. Olshansky et al. *(95)* used the term "manufactured time" to describe use of prescription drugs and other methods to increase the odds of living beyond our reproductive years. Therefore, it should not be surprising that micronutrient supplements may be particularly beneficial to the immune and other organ systems of older individuals.

Goodwin *(96)* suggested that the relationship between depressed cellular immune function and subsequent increased mortality may result from compromised immunity as a marker for clinically latent diseases or poor overall physiological function. However, impaired immunity may also contribute to a reduced ability to defend against infections, cancers, and, perhaps, cardiovascular heart disease.

5. RESEARCH NEEDED ON MICRONUTRIENTS AND IMMUNITY IN OLDER PEOPLE

As previously discussed, several cross-sectional studies have been performed in the last 12 yr that assess relationships between micronutrient nutrition and immunity *(49,50)*. Generally, significant associations between serum micronutrient concentrations or use of micronutrient supplements and various measures of immunity were found in some studies but not others *(49–52)*. However, these studies compared micronutrient supplement users with nonusers but did not evaluate use of specific supplements, and it is likely that some individual or combinations of micronutrients can improve immunity and others cannot.

The clinical trials performed to date regarding micronutrient supplementation and immunity usually have involved healthy older subjects consuming their usual diets. In the case of some single nutrient studies, subjects lived in metabolic units and consumed standardized meals that contained about the RDA of all essential micronutrients. It is possible that the improvement in immunity found in some studies was a result of correction of underlying deficiencies. However, it is also likely that micronutrient supplements enhance immunity even in the absence of underlying deficiencies, at least based on current concepts of "deficiency." This should not be surprising, because optimal immune function was not a factor in establishment of the current Dietary Reference Intakes (DRIs) or in defining laboratory normal ranges for circulating micronutrient concentrations. In fact, daily intakes that optimize immunity may differ from both the current DRIs and intakes that may prevent chronic diseases. For example, the current DRI/RDA for vitamin E (equivalents of 15 mg of α-tocopherol for adult females and males) is substantially lower than amounts that optimize immune functions or are associated with a reduced risk of cardiovascular heart disease *(64,65,97,98)*. Similarly, the current RDA for vitamin C is adequate to prevent development of scorbutic lesions but appears to be less than the intake that could optimize immunity or provide other health benefits *(4,99)*. Recommendations for an optimal intake of any micronutrient need to balance the impact of that nutrient on various health outcomes as well as consider possible adverse effects of relatively high doses.

New studies are needed, particularly those that focus on clinical outcomes and have considerable statistical power. An example is the recent investigation of Graat et al. *(100)*. They conducted a randomized, double-blind, placebo-controlled trial of 652 Dutch men and women older than age 60 yr who were given either a placebo, multivitamin/mineral supplement, 200 mg of vitamin E as α-tocopherol, or both supplements. The multivitamin/mineral supplement included 24 essential micronutrients and one "possibly essential" trace element (silicon). The primary outcome measures were the incidence and severity of acute respiratory tract infections. The mean duration of participation was 441 d, and 84% of subjects were compliant with the protocol. The incidence of acute respiratory tract infections did not differ significantly among treatment groups. Surprisingly, infection severity (measured as duration of infection, restriction of activities, number of symptoms, and presence of fever) actually increased significantly ($p = 0.03–0.009$) in the groups ingesting vitamin E supplements. This study focused on a clinical outcome but did not include laboratory evaluation of immunity. Therefore, immune system assays that might explain the study results were not available. Only 0.2% of study subjects had low plasma α-tocopherol concentrations at enrollment, and this may have precluded a beneficial effect of vitamin E supplements. The adverse effects on infection severity may have resulted from the long duration of high-dose supplementation in a cohort with normal plasma α-tocopherol concentrations at enrollment and was consistent with previously cited *(59,60)* studies on β-carotene that demonstrated adverse effects after long-term high-dose supplementation. These studies suggest caution in the long-term use of high-dose single micronutrients.

There is considerable evidence that patterns of physical activity and exercise can influence immunity both acutely and chronically; however, very few studies have addressed interactions among physical activity, immunity, and micronutrient nutrition.

It should be emphasized that the potential of micronutrient supplements to improve immunity or exert other beneficial effects must be considered in relation to their consumption from food. This is especially true for low- to moderate-dose supplements, for which the intake from food and supplements may be similar. Clearly, supplement use should be encouraged in conjunction with a sound diet that emphasizes fruits, vegetables, whole grains, and other sources of micronutrients and limits the intake of saturated fats. However, it is likely that beneficial intakes of some nutrients, such as vitamin E, may not be possible from a relatively low-fat diet in the absence of supplement use.

The promising but variable results of studies performed to date suggest that continued research is needed regarding nutrition and immunity in older people. Such efforts should include the following:

1. A focus on long-term, placebo-controlled, double-blind clinical trials and prospective epidemiological studies that have sufficient statistical power.
2. Study of interactions among physical activity/exercise patterns, immunity, and nutrition.
3. Evaluation of effects of nutrition on both humoral (e.g., antibody responses to vaccination) and cellular (e.g., DHST responses) immunity using clinically relevant assays and on clinical outcomes (e.g., infectious disease incidence, duration, and severity).
4. Evaluation of dietary modification alone or in combination with low doses of supplemental micronutrients. Studies of older individuals consuming their usual diets are also needed.

5. Long-term studies that address the persistence of the effects of micronutrients on immunity both during and after micronutrient supplementation.
6. Use of appropriate inclusion and exclusion criteria in identification of subjects for study.
7. Study of both single micronutrients and multivitamin/minerals, with a focus on the antioxidant micronutrients and other widely used single or multiple micronutrient supplements.
8. Identification of host-specific factors (e.g., gender, age) that influence micronutrient —immunity interactions as well as the basis for these effects.
9. Identification of the molecular mechanisms and genes that determine the effects of micronutrients on immunity. This will become increasingly important as new genes that influence aging are identified.

About 100 million Americans (approx 40% of the population) take multivitamin/mineral supplements either alone or in combination with higher doses of the antioxidant vitamins *(78,79)*. Well-designed studies that assess the health impacts of this practice are urgently needed and should include evaluation of effects on the immune system.

6. RECOMMENDATIONS

Physicians and other health care providers should advise their patients to eat diets low in saturated fat and high in fruits and vegetables. This will ensure consumption of significant quantities of the micronutrients (and phytochemicals) that can favorably affect immunity. Additionally, older subjects should be encouraged to take a low-dose multivitamin/mineral supplement, especially those with poor diets. Higher daily doses of the antioxidant micronutrients vitamin C (200–500 mg) and vitamin E (200–400 IU) may also be appropriate for some, but not all, older individuals. Taking high supplemental doses of other micronutrients that can adversely affect immunity (e.g., zinc) should be persuasively discouraged. High doses of supplemental β-carotene are not recommended for smokers because of their association with the development of lung cancer and are unwise for other people, as recently concluded by the US Preventive Services Task Force *(101)* The favorable effects of regular exercise on immunity also should be mentioned to patients. This advice (low-fat diet, high intake of fruits and vegetables, regular exercise, and supplemental vitamins) may not only promote optimal immunity but is also likely to reduce the risk of cardiovascular heart disease and some cancers.

In a widely publicized article, Fletcher and Fairfield *(102)* reviewed the health effects of vitamin supplements and concluded, "All adults (should) take one multivitamin daily." They based this recommendation on beneficial health effects other than effects on immunity. The likely beneficial impact on immunity in older people, as discussed in this chapter and another recent review *(103)*, is an additional benefit that further supports this recommendation.

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23 Vitamin A and the Prevention of Morbidity, Mortality, and Blindness

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KEY POINTS

- Vitamin A deficiency remains a leading cause of morbidity, mortality, and blindness among preschool children in developing countries worldwide.
- Consequences of vitamin A deficiency include impaired immune function, growth retardation, anemia, xerophthalmia, and blindness.
- The impaired immunity resulting from vitamin A deficiency results in increased morbidity and mortality from some infectious diseases such as measles, diarrheal disease, and human immunodeficiency virus infection.
- Periodic high-dose vitamin A capsule distribution has been implemented by some developing countries as a short-term strategy to prevent vitamin A deficiency among preschool children.
- Diverse long-term strategies, including nutrition education, food fortification, and homestead food production, are needed to prevent vitamin A deficiency in developing countries. Ultimately, family-based approaches are required to address vitamin A deficiency, because pregnant women and women of childbearing age are at high risk of vitamin A deficiency in many countries and are not reached by vertical programs such as vitamin A capsule distribution.

1. INTRODUCTION

Vitamin A deficiency is a major cause of morbidity, mortality, and blindness among infants and young children and a considerable cause of morbidity among pregnant women and women of childbearing age in developing countries worldwide. Vitamin A deficiency occurs when the dietary intake of vitamin A is inadequate to meet the metabolic demands for vitamin A. Periods of rapid growth, inflammation, malabsorption, and episodes of infection can increase the need for vitamin A. The main dietary sources of vitamin A include liver; butter, egg yolk, and dairy products; and dark green leafy vegetables, yellow fruits, and carrots. Vitamin A deficiency is characterized by impaired immune function, growth retardation, anemia, and xerophthalmia, and the immunosuppression of

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vitamin A deficiency is associated with increased morbidity and mortality from some infectious diseases. As early as the 1920s and 1930s, improved dietary vitamin A intake or vitamin A supplementation was shown to reduce mortality in children and reduce morbidity in pregnant women *(1,2)*. Improving vitamin A intake, whether through improved diet, fortification of foods, or periodic supplementation, is expected to reduce morbidity and mortality for millions of children and perhaps women of childbearing age, and there may be many other therapeutic applications for vitamin A that have not yet been realized. This chapter provides health professionals with a concise review of the epidemiology, pathophysiology, diagnosis, treatment, and prevention of vitamin A deficiency.

2. METABOLISM OF VITAMIN A

2.1. Absorption of Vitamin A

Vitamin A, or all-*trans* retinol, is a fat-soluble substance found in animal foods and dairy products. Vitamin A is available as preformed vitamin A (contained in high concentrations in such foods as liver, cod liver oil, butter, or egg yolks) or as provitamin A carotenoids (found in dark green leafy vegetables, carrots, papaya, and mangos). Retinyl esters contained in animal foods are hydrolyzed to retinol in the intestinal lumen by pancreatic triglyceride lipase and phospholipase B *(3)*. Free retinol is taken up by enterocytes in a process that may involve both diffusion and protein-mediated facilitated transport *(3)*. In the enterocyte, retinol is complexed with cellular retinol-binding protein type 2 and is then re-esterified by the enzyme lecithin:retinol acyltransferase *(3,4)*. The retinyl esters are incorporated into chylomicrons and carried to the liver via the lymphatic circulation *(5)*. Provitamin A carotenoids such as β-carotene may be converted to retinaldehyde through cleavage by carotenoid-15,15′-dioxygenase or by an excentric cleavage pathway. Approximately 50 of more than 600 carotenoids found in nature may be converted to vitamin A *(6)*. The bioavailability of provitamin A carotenoids is much less than that of preformed vitamin A for a variety of reasons, including differences in efficacy of absorption, the plant matrix, and biochemical conversion *(7)*. Although carotenoids, such as β-carotene, and vitamin A often are popularly regarded to be equivalent, there may be large differences in biological functions of these two nutrients. Provitamin A carotenoids have many metabolic products that result from excentric cleavage; therefore, a large portion of provitamin A carotenoids are not simply metabolized to vitamin A. In relation to antioxidant properties, vitamin A is a less potent antioxidant than β-carotene.

2.2. Storage and Transport of Vitamin A

Approximately 90% of the vitamin A in the body is stored in the liver as retinyl esters *(8)*. The liver has the capacity to store enough vitamin A to last for several months, with longer storage capacity among adults than children. Periodic high doses of vitamin A are largely effective in preventing vitamin A deficiency for long periods because of the liver's ability to store vitamin A *(9)*. Retinol is released from the liver in combination with plasma retinol-binding protein (RBP) and transthyretin (TTR). Retinol is poorly soluble in water and is carried in the blood sequestered inside the carrier proteins RBP and TTR. Retinol appears to enter cells via specific receptors, although it is unclear whether all cells contain these receptors *(10)*.

2.3. The Retinoid Receptors

Vitamin A exerts its effects through retinoid receptors found in the nucleus of the cell that are members of the nuclear hormone receptor superfamily *(11)*. These receptors resemble steroid and thyroid hormone receptors. In the cell, retinol is converted to its active metabolite, all-*trans*-retinoic acid. Retinoic acid influences gene activation through specific receptors that belong to the superfamily of thyroid and steroid receptors *(11)*. Retinoic acid receptors (RARs) act as transcriptional activators for specific target genes. The RAR is expressed as several isoforms, referred to as RAR α, β, and γ; retinoid-x receptor (RXR) is also expressed as several isoforms, referred to as RXR α, β, and γ *(12)*. All-*trans* retinoic acid is a high-affinity ligand for RARs, and 9-*cis*-retinoic acid is a high-affinity ligand primarily for RXRs, but it can also bind with RARs. 9-*cis*-retinoic acid seems to be functionally distinct from all-*trans*-retinoic acid, and interconversion may exist between the two isomers. Each RAR and RXR has a specific DNA-binding domain by which these nuclear receptors may affect transcriptional activity. The DNA sequences required for action by RAR and RXR are known as retinoic acid response elements (RAREs). RAR and RXR receptors form heterodimers (RAR/RXR) that bind to DNA and control gene activity *(13)*. In addition, RXR receptors can form heterodimers with the thyroid hormone receptor, vitamin D_3 receptor (VDR), peroxisome proliferator-activated receptors (PPAR), and a number of orphan nuclear receptors. Most RAREs seem to occur in the regulatory region of genes. In the presence of 9-*cis*-retinoic acid, RXR/RXR homodimers may form and recognize a subset of RAREs or inhibit the formation of certain heterodimers.

Orphan nuclear receptors (gene products that embody structural features of nuclear receptors that were identified without any prior knowledge of their association with a putative ligand) *(14)*, such as chicken ovalbumin upstream promoter transcription factor *(15)*, apolipoprotein AI regulatory protein-1, transforming growth factor-β-activated kinase 1 (TAK1) *(16)*, RVR *(17)*, retinoid Z receptor *(18)*, retinoid-related orphan receptors *(19)*, and thymus orphan receptor *(20)* may repress or modulate the induction of genes by retinoic acid. Thus, RARs and RXRs may interact with multiple transcriptional mediators and/or corepressors, adding an enormous level of complexity to regulation of retinoic acid responses *(21)*. About 40 vertebrate orphan nuclear receptors have been identified *(14)*. In the scientific literature, the number of genes regulated either directly or indirectly by retinoids is growing rapidly *(22)*. Other vitamin A metabolites in the retroretinoid family may support biological functions via a pathway that is distinct from the retinoic acid pathway. 14-hydroxy-4,14-*retro* retinol supports cell growth, whereas anhydroretinol inhibits cell growth *(23–25)* and induces cell death *(26)*. Additionally, the oxoretinoids may play a role as RAR ligands *(27)*.

Recently described nuclear receptor-associated proteins, such as SMRT/N-CoR, Sin3, and histone deacetylases-1 and -2), and histone acetylases (CBP/p300 and P/CAF), appear to function as corepressors and coactivators of transcription. A binary paradigm has emerged as an attempt to explain how these proteins work. Unligated receptors bind to response elements of target genes and repress transcription through recruitment of a repressor complex containing corepressors. Ligand binding causes the dissociation of corepressor proteins and promotes the association of coactivators with liganded receptors. The formation of these regulatory complexes may prove critical in determining which signaling pathway is followed at a given time *(28)*.

3. EFFECTS OF VITAMIN A DEFICIENCY

3.1. Immunity

3.1.1. Infectious Diseases

Clinical vitamin A deficiency and/or low circulating vitamin A concentrations consistent with vitamin A deficiency have been described in a variety of infectious diseases, including chicken pox *(29)*, diarrhea *(30)*, endometritis *(31)*, human immunodeficiency virus (HIV) infection *(32)*, leprosy *(33)*, malaria *(34)*, measles *(35)*, meningococcal disease *(36)*, otitis media *(37)*, pneumonia *(38)*, respiratory syncytial virus infection *(39)*, rheumatic fever *(40)*, tuberculosis *(41)*, schistosomiasis *(42)*, and whooping cough *(43)*. Serum retinol concentrations may drop during infection because of decreased intake and absorption of dietary vitamin A caused by diarrhea or intestinal pathogens, decreased mobilization of hepatic reserves of retinol, accelerated utilization by target tissues, and increased urinary losses of vitamin A during the acute phase response *(29,44,45)*. Overall, episodes of infection seem to hasten the depletion of vitamin A stores *(29,46,47)*. Given the close relationship between immune effector cell function and the availability of vitamin A, low serum vitamin A concentrations during infection may have detrimental effects on the immune response. Infectious diseases that have been associated with vitamin A deficiency are shown in Table 1.

Low serum vitamin A concentrations have been associated with night blindness and increased infectious disease morbidity *(48)*. Individuals with low vitamin A concentrations have increased risk of morbidity and mortality during measles *(35)*, HIV infection *(32,49)*, respiratory syncytial virus infection *(39)*, and meningococcal disease *(36)*. Impairment of immune responses during infections may be related to the inability of T and B cells to function without adequate levels of retinol *(50)*. Night blindness in pregnant women has been associated with increased infectious disease morbidity and elevated acute phase proteins *(48,51)*.

3.1.2. Increased Mortality

The biological consequences of vitamin A deficiency during infection include increased morbidity and mortality *(52)*. After an extensive global survey of vitamin A deficiency, in 1964, Oomen et al. recognized that there was a vicious cycle of vitamin A deficiency and infection: "Not only may deficiency of vitamin A itself play an important role in lowering the resistance to infection...but infectious diseases themselves predispose to and actually precipitate xerophthalmia" *(53)*. The idea of a "vicious cycle" was revived again in the late 1980s *(54)*. Longitudinal studies in Indonesia showed that the mortality rate for children with mild xerophthalmia was four times greater than age-matched controls. The higher mortality was apparent even after accounting for differences in concurrent illnesses and protein-energy status. In addition, children with pre-existing xerophthalmia had higher rates of subsequent diarrhea and respiratory disease, whereas children who developed diarrhea and respiratory disease were at increased risk of subsequently developing xerophthalmia *(55,56)*. Other studies also showed an association between mild vitamin A deficiency and increased morbidity in children *(57,58)*.

3.1.3. Mucosal Immunity

Vitamin A deficiency may cause a breakdown in mucosal immunity through pathological changes in the epithelia of respiratory, gastrointestinal, and urinary systems.

Table 1
Infectious Diseases Associated With Vitamin A Deficiency

Disease	*Subjects*	*References*
Chicken pox	Children	*29*
Diarrhea	Children	*30*
Endometritis	Adults	*31*
Human immunodeficiency virus type 1	Adults	*32*
Leprosy	Adults	*33*
Malaria	Children	*34*
Measles	Children	*35*
Meningococcal disease	Children	*36*
Otitis media	Children	*37*
Pneumonia	Children, adults	*30, 38*
Respiratory syncytial virus	Children	*39*
Rheumatic fever	Children	*40*
Tuberculosis	Children	*41*
Schistosomiasis	Adults	*42*

This includes keratinizing metaplasia and loss of goblet cells and mucus *(50,59)*. Loss of intestinal brush border and goblet cells and squamous metaplasia as well as destruction of ciliated epithelia in the respiratory system have been reported in vitamin A-deficient animals. Decreased levels of secretory immunoglobulin (Ig)A in saliva have been reported in vitamin A-deficient children. Animal models suggest that vitamin A deficiency is associated with lower total IgA concentrations in the gut *(60)* and impaired antigen-specific IgA responses *(61–63)*.

3.1.4. Cellular Immunity

Atrophy of the thymus, spleen, and lymphoid tissues has been observed in children who died with vitamin A deficiency *(50,59)*. Mild vitamin A deficiency is associated with underlying alterations in circulating T-cell subpopulations, such as decreased $CD4^+CD45RA^+$ T cells (or "naïve" CD4 T cells), and decreased CD4/CD8 ratios *(64)*. During HIV-1 infection, vitamin A-deficient adults had lower circulating numbers of $CD4^+$ lymphocyte compared to adults with normal vitamin A levels. Lower plasma vitamin A concentrations also are associated with alterations of T-cell subpopulations during acute meningococcal infection in children and during HIV-1 infection in adults *(36,65)*.

Vitamin A and its metabolites, including T cells of the thymus, lymphocytes in lymphoid tissue, and peripheral blood lymphocytes, are essential for immune effector cell function *(66)*. Retinoic acid is involved in the expression of interleukin (IL)-2 receptor on T cells. Retinol is essential for growth and differentiation of B cells and the production of antibodies *(67,68)*. Retinoic acid may enhance immunoglobulin synthesis by cord blood mononuclear cells through increased T-cell help, that is, modulation of cytokines, which induce B cells to differentiate into greater numbers of immunoglobulin-secreting cells *(69)*. In vitro studies suggest that vitamin A modulates growth and function of T and B cells either directly or through its active metabolite, all-*trans* retinoic acid.

Vitamin A and β-carotene supplementation are associated with changes in lymphocyte numbers and T-cell subpopulations. The depression in circulating lymphocytes following surgery has been reversed in adults by administration of high-dose vitamin A *(50)*.

Children with clinical and subclinical vitamin A deficiency who received vitamin A had increases in circulating $CD4^+$ lymphocytes, especially $CD4^+CD45RA^+$, and an increase in CD4/CD8 ratio 5 wk after dosing *(64)*. Measles infection is characterized by immune suppression, leukopenia, and decreased circulating $CD4^+$ lymphocytes. Administration of high-dose vitamin A to children with measles has been shown to increase total circulating lymphocytes and enhance IgG responses to measles *(70)*. Vitamin A and related metabolites are potent enhancers of cellular differentiation, and vitamin A-related differentiation of lymphocytes may be a possible mechanism for the increase in $CD4^+$ lymphocytes.

3.1.5. Antibody Responses

A hallmark of vitamin A deficiency is the impaired ability to mount an antibody response against T cell-dependent protein antigens *(50,65,71)*. Other types of immune responses may be unaffected by vitamin A deficiency *(71,72)*. The antibody response to immunization with tetanus toxoid or other antigens has been used to examine immune competence. Vitamin A-deficient children in Indonesia showed reduced antibody responses to tetanus toxoid *(73)*. Supplementation with high-dose vitamin A (200,000 IU or the equivalent of 60 mg of retinol) was associated with a more than twofold enhancement of both primary and secondary IgG responses to tetanus toxoid compared to children who received placebo. Vitamin A potentiated IgG_1 subclass responses to tetanus toxoid, which is the subclass usually involved in the protective antibody response to tetanus *(50)*. These findings suggest that the administration of vitamin A or related retinoids improves the ability to mount an IgG response to T cell-dependent antigens. Vitamin A supplementation given simultaneously with childhood immunization contacts enhanced antibody responses to diphtheria vaccine in infants younger than age 6 mo *(74)*. Vitamin A supplementation did not appear to interfere with antibody responses to live trivalent oral polio vaccine when given to infants in India *(75)* and Indonesia *(76)*.

3.2. Growth

Vitamin A deficiency is characterized by both stunting and wasting *(77)*. This type of relationship is not surprising, because the gene for growth hormone is activated by retinoic acid *(78)*. However, the reported effects of vitamin A supplementation on child growth are not consistent *(79)*. A randomized controlled clinical trial involving more than 2000 preschool children in Indonesia demonstrated that vitamin A supplementation had a selective effect on ponderal growth in boys but not girls *(79)*. Improved linear growth among both boys and girls was noted in a controlled trial of vitamin A-fortified monosodium glutamate in more than 1600 children *(80)*. Another trial of vitamin A supplementation showed no effect on growth in 592 preschool children, but this small sample size may have lacked sufficient power to examine the impact of vitamin A on growth *(81)*. Vitamin A supplementation appeared to improve linear but not ponderal growth in a controlled clinical trial in Indonesia that studied 1047 preschool children *(82)*.

3.3. Reproduction and Pregnancy

Vitamin A is essential for normal reproduction *(83)*. Animals deficient in vitamin A are unable to produce sperm, and vitamin A deficiency also may affect fertility in the female. In both human and animal models, vitamin A deficiency is associated with increased placental infections. Pregnancy increases the risk of vitamin A deficiency for

both mother and newborn. Epidemics of night blindness among pregnant women were well-known in Europe in the early part of this century *(84)*, and night blindness is so common in some cultures in developing countries that it has been considered to be a normal associate of pregnancy, similarly to morning sickness. Maternal vitamin A status may be important to birth outcome, because increased mortality and low birth weight have been reported among HIV-infected pregnant women with low serum vitamin A levels during pregnancy *(85)*. Low serum vitamin A concentrations in pregnant women (who are confirmed HIV-negative) also have been associated with increased infant mortality *(86)*. A recent clinical trial showed that the infants of women who received vitamin A supplementation (10,000 IU/d) during pregnancy had an increase in birth weight *(87)*.

3.4. Hematopoiesis

Vitamin A deficiency is associated with anemia in children and adults *(88)*. Vitamin A appears to be involved in the pathogenesis of anemia through diverse biological mechanisms, such as the enhancement of growth and differentiation of erythrocyte progenitor cells, potentiation of immunity to infection and reduction of the anemia of infection, and mobilization of iron stores from tissues *(89)*. Vitamin A supplementation or fortification has been shown to improve iron status and reduce anemia among children and pregnant women *(89,90)*. The combination of vitamin A and iron in a single supplement appears to reduce anemia more effectively than either iron or vitamin A alone *(89,90)*. Studies in knockout mice have suggested that RAR activity is not necessary for normal hematopoiesis; however, all-*trans* retinoic acid and RARs play a role in modifying hematopoiesis *(91)*.

3.5. Vision

Vitamin A deficiency is the leading cause of blindness among children worldwide. The term "xerophthalmia" is used to describe the wide spectrum of eye disease associated with vitamin A deficiency, and "keratomalacia" refers to the most severe stage, in which the cornea undergoes ulceration and often suffers blindness *(92)*. Vitamin A is essential for generation of rhodopsin, a visual pigment necessary for vision. The earliest clinical manifestation of vitamin A deficiency is night blindness *(92)*. Vitamin A deficiency generally has an effect on mucosal epithelia, including the conjunctiva and cornea. Mild vitamin A deficiency causes squamous metaplasia of the conjunctiva (which may be detectable microscopically) or may form a Bitot's spot (a well-demarcated area of keratinizing squamous metaplasia on the temporal or nasal bulbar conjunctiva). Bitot's spots are considered pathognomonic for mild vitamin A deficiency. Severe xerophthalmia is characterized by classic, punched-out, full-thickness corneal ulceration; these ulcers may be sterile or may rapidly become secondarily infected with melting of the corneal stroma. The ocular manifestations of vitamin A deficiency are described in detail in refs. *30*, *45*, and *92*.

4. EPIDEMIOLOGY OF VITAMIN A DEFICIENCY

4.1. Prevalence

The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) estimate that there are 2.8 million preschool children with clinical vitamin A deficiency and 251 million preschool children with subclinical vitamin A deficiency

worldwide *(93)*. Another recent estimate suggests that there are 4.4 million preschool children with xerophthalmia and 127 million preschool children with serum retinol concentrations of less than 0.70 μmol/L or abnormal impression cytology *(94)*. An estimated 7.2 million pregnant women are deficient in vitamin A (defined as having serum or breast milk vitamin A concentrations less than 0.70 μmol/L) *(94)*. An estimated 6 million pregnant women develop night blindness each year *(94)*. The risk of vitamin A deficiency is highest among populations in south Asia, southeast Asia, sub-Saharan Africa, parts of Central and South America, and some areas of the Western Pacific *(94,95)*.

The global prevalence of blindness resulting from vitamin A deficiency is not well-known. Much of the blindness from vitamin A deficiency occurs in children with acute, complicated measles, and in surveys from schools for the blind or visually impaired and in community-based surveys, it is often not possible to distinguish between corneal scarring resulting from vitamin A deficiency alone or corneal scarring from vitamin A deficiency combined with measles; often, this diagnosis is combined *(96)*. Measles blindness is the single leading cause of blindness among children in low-income countries, accounting for an estimated 15,000 to 60,000 cases of blindness per year *(96)*, and the blindness that occurs with measles can be prevented with high-dose vitamin A supplementation. Worldwide, vitamin A capsule distribution programs and other improvements in sanitation and hygiene may be reducing the prevalence of xerophthalmia *(97,98)*.

4.2. Risk Factors for Vitamin A Deficiency

Preschool children, pregnant women, and nonpregnant women of childbearing age are at highest risk for vitamin A deficiency. Among preschool children, boys are at higher risk than girls *(99–102)*. Early weaning or not breastfeeding are risk factors for vitamin A deficiency among infants in developing countries *(101,103–105)*, because the main source of vitamin A for young infants is the retinol contained in breast milk. Vitamin A deficiency tends to occur among the poorest families; therefore, low socioeconomic status is a strong risk factor for vitamin A deficiency. Concomitant protein-energy malnutrition also is a risk factor for vitamin A deficiency *(101,103)*. The risk of vitamin A deficiency can increase with the change of seasons in some areas, with the highest risk corresponding to the lowest availability of dietary vitamin A *(98)*.

5. DIAGNOSIS OF VITAMIN A DEFICIENCY

5.1. Eye Signs and Symptoms

Although vitamin A deficiency has a wide spectrum of clinical manifestations (Table 2), the clinical diagnosis in children is most commonly made through recognition of ocular signs and symptoms, including night blindness, Bitot's spots, corneal xerosis, or corneal ulceration/keratomalacia *(92)*. The detection of night blindness and/or Bitot's spots is often useful for determining the prevalence of mild xerophthalmia among children in developing countries. Among pregnant women in developing countries, assessment of night blindness is an important screening test for vitamin A deficiency. Night blindness also can be used to diagnose vitamin A deficiency among nonpregnant women of childbearing age *(106)*.

5.2. Serum Retinol Concentrations

Serum vitamin A concentrations remain the most widely used indicator of vitamin A status *(107)* and are especially useful in large epidemiological studies. Vitamin A levels less

Table 2
Clinical Features of Vitamin A Deficiency

Organ system	*Clinical features*
Eye	Night blindness, Bitot's spots, corneal xerosis, corneal ulcers, fundus xerophthalmicus, keratomalacia, blindness.
Immune system	Atrophy of thymus, lymph nodes, spleen; depressed antibody and cell-mediated responses, T-cell subset alterations, increased morbidity and mortality.
Hematological	Anemia, impaired lymphopoiesis.
Lungs	Loss of ciliated epithelium, squamous metaplasia, goblet cells, and mucus; increased pulmonary infections.
Gastrointestinal tract	Loss of brush border, atrophy of Peyer's patches, loss of goblet cells and mucus, greater severity of diarrhea.
Genitourinary	Squamous metaplasia of bladder epithelium, increased infections.
Reproductive	Reproductive failure, low birth weight, placental infections.
Growth	Stunting, wasting.

than 0.7 μmol/L in children, and less than 1.05 μmol/L in adults are considered to be consistent with vitamin A deficiency. Factors contributing to low serum vitamin A concentrations include poor intake and absorption, inadequate liver stores, liver disease, increased utilization, and the acute phase response. During the acute phase response, RBP may dissociate from the RBP–transthyretin–retinol complex in circulating blood, allowing abnormal vitamin A losses in the urine and decreased circulating vitamin A levels *(44)*. High urinary losses of vitamin A have been reported in individuals with pneumonia, nephritis, tuberculosis, sepsis, and malaria *(44)*. Biological function may be compromised when serum vitamin A concentrations fall below 1.05 μmol/L *(107,108)*; however, further studies are needed to determine whether threshold effects exist in human vitamin A deficiency.

The measurement of acute phase proteins, such as C-reactive protein and α_1-acid glycoprotein, has been advocated to improve the interpretation of serum vitamin A levels in populations with a high prevalence of infection *(109)*. Elevated acute phase proteins and infections are more common in pregnant women with night blindness *(48)* and in preschool children with night blindness and/or Bitot's spots *(110)*. These studies show that clinical vitamin A deficiency, infections, elevated acute phase proteins, and low serum vitamin A concentrations are closely associated. Although acute phase proteins do not provide any mutually exclusive information about whether low serum vitamin A concentrations are the result of true vitamin A deficiency or an acute phase response in an individual with inflammation or infection, the argument has been made that acute phase proteins can identify those individuals with low serum retinol concentrations who are "misclassified" as vitamin A-deficient *(111)*.

5.3. Breast Milk Retinol Concentrations

Breast milk vitamin A concentrations have been advocated as an indicator of vitamin A status in women because the technique is fairly simple, is noninvasive, and is considered

more responsive to dietary or supplementary vitamin A than other indicators *(112)*. The presence of vitamin A in human milk has been established for nearly eight decades *(113)* and has been considered to reflect maternal dietary intake of vitamin A *(114,115)*. Supplementation of both pregnant women *(116)* and lactating women *(117)* with oral vitamin A increases breast milk vitamin A concentrations. The response of breast milk vitamin A concentrations following oral vitamin A supplementation has been characterized among pregnant women with a high prevalence of infections *(118)*. Breast milk vitamin A concentrations of less than 0.70 μmol/L and 1.00 μmol/L have been proposed as cut-offs for low levels of vitamin A in breast milk *(112)*.

5.4. Other Methods of Diagnosis

Vitamin A status can be measured by other techniques that measure end organ effects of vitamin A (conjunctival impression cytology, dark adaptation) and liver reserves of vitamin A (relative dose—response [RDR], modified relative dose—response [MRDR]). Conjunctival impression cytology, a technique proposed as early as the 1950s *(119,120)*, is based on cytological examination of cells removed from the conjunctiva of the eye *(121)*. Dark adaptation has been recognized for decades to be useful, in various forms, for measuring vitamin A deficiency *(122)*. The RDR *(123)* and the MRDR tests *(124)* are indirect measures of hepatic vitamin A stores. Generally, abnormal RDRs and MRDRs correlate well with low serum vitamin A concentrations. MRDR may have limited sensitivity in individuals with malnutrition *(125)*. Another technique, which originally was proposed in the 1940s, involves the measurement of pupillary diameter. This technique has advantages of being simple, rapid, and noninvasive, and early results have been promising *(126,127)*. The molar ratio of RBP to TTR has been proposed as a method to diagnose vitamin A deficiency during inflammation *(128)*. However, the molar ratio of RBP to TTR was not useful for diagnosing vitamin A deficiency in a large study of hospitalized children with infections *(129)* and was shown to have poor sensitivity and specificity as a tool for diagnosis of vitamin A deficiency *(130)*.

6. OTHER

6.1. Vitamin A and Bone Fractures

Higher intake of retinol (> 1500 μg/d compared with < 500 μg/d) has been associated with an increased risk of hip fracture in older adults in Northern Europe *(131)*. For comparison, in the Third National Health and Nutrition Examination Survey (NHANES III) in 1988–1994, the 90th percentiles of total retinol intake from foods for men and women ages 51 to 70 yr were 1523 μg/d and 1281 μg/d, respectively *(132)*. Median intake of vitamin A in Scandinavian countries is reportedly higher than in Southern Europe, and a cross-sectional study of 175 women suggested that the risk of hip fracture increased by 68% for every 1000-μg increase in daily intake of vitamin A *(131)*. In the Nurses' Health Study in the United States, women in the highest quintile of total vitamin A intake (approx 3000 μg/d of retinol equivalents) had an increased relative risk (RR) of hip fracture (RR = 1.48, 95% confidence interval [CI] 1.05–2.07) *(133)*. However, potential confounding may exist in the Nurses' Health Study, because moderate alcohol consumption has been associated with a lower risk of hip fracture, and women with higher vitamin A intake consumed less alcohol *(134)* (*see* Chapter 28).

In a population-based longitudinal study of 2322 men ages 49 to 51 yr, serum retinol concentrations in the highest quintile (>2.64 μmol/L) were compared with the middle quintile (2.17–2.36 μmol/L), and men in the highest quintile had an increased RR of fracture (RR = 1.64, 95% CI, 1.12–2.41) *(135)*. In NHANES III, serum retinol concentrations at the 75th percentile for non-Hispanic Caucasian men and women ages 51–70 yr were 2.55 and 2.37 μmol/L, respectively; for non-Hispanic African-American men and women ages 51–70 yr, retinol concentrations were 2.44 and 2.27 μmol/L, respectively *(136)*. Serum retinol concentrations greater than 2.64 μmol/L (as reported in Sweden; ref. *135*) are not exceptionally high, and even the upper limit of normal is 3.5 μmol/L *(137)*. The association between retinol intake and levels has not been corroborated by other large studies. In NHANES III, there was no association between serum retinyl esters and bone mineral density *(138)*. Additionally, a recent randomized trial involving 80 healthy men ages 18–58 yr showed that vitamin A supplementation (25,000 IU/d) did not alter serum markers of skeletal bone turnover *(139)*.

7. VITAMIN A AS A PUBLIC HEALTH INTERVENTION

7.1. Brief History

The use of liver extracts as a cure for night blindness has been described in antiquity. In the 19th century, cod liver oil—a potent source of vitamins A and D—was empirically recognized to have therapeutic value in the treatment of anemia, tuberculosis, and other disorders. In the 18th and 19th centuries, there were numerous reports associating night blindness with increased morbidity and mortality. The existence of vitamin A was demonstrated through a long incremental process that included contributions by Magendie *(140)*; Lunin *(141)*; Socin *(142)*; Pekelharing *(143)*; Stepp *(144)*; Hopkins *(145)*; Osborne and Mendel *(146,147)*; McCollum, Davis, Simmonds, Becker, and Shipley *(148,149)*; Karrer *(150)*; Holmes and Corbett *(151)*; and others. Different investigators claimed to have "discovered" vitamin A, but the development of scientific thought occurred only through many cumulative steps and separate contributions by many individuals *(152)*.

By the 1920s, increased susceptibility to infection and high child mortality were attributed to the lack of vitamin A *(153)*, and provision of vitamin A was shown to reduce child mortality *(43)*. In 1924, Erik Widmark, a professor at the University of Lund, concluded, "There must be in a population in which xerophthalmia occurs a much larger number of cases in which the deficiency in vitamin A, without producing the eye disease, is the cause of a diminished resistance to infections," *(154)*. Nutritional deprivation experiments suggested that vitamin A deficiency increased the risk of infections, and in 1928, vitamin A was termed the "anti-infective" vitamin *(155)*. In the 1920s and 1930s, at least 30 trials were conducted to determine whether vitamin A could reduce the morbidity and mortality from respiratory infections, measles, puerperal sepsis, tuberculosis, and other infections *(156)*. These early trials did not provide definitive information regarding the use of vitamin A for infections, with the exception of the important discovery by Joseph Ellison that vitamin A supplementation reduced mortality in children with measles *(2,157)*. Scientific interest in use of vitamin A as anti-infective therapy appeared to wane with the advent of the sulfa antibiotics and improvements in diet in industrialized countries in the late 1930s. The League of Nations recognized that vitamin A deficiency contributed to higher mortality rates from infectious diseases, and public health programs

were aimed at improving vitamin A status to reduce infectious disease morbidity and mortality *(1,158)*.

7.2. Recent Clinical Trials of Vitamin A for Reducing Infections and Mortality

In studies conducted in the 1970s, the association of xerophthalmia with mortality again was observed among preschool children in Indonesia *(52)*. A large community-based trial demonstrated that regular vitamin A supplementation reduced childhood mortality by 34% in Indonesia *(159)*. The Indonesian study was followed by a large series of clinical trials in Indonesia, India, Nepal, Sudan, and Ghana *(80,160–166)*. These studies demonstrated that periodic vitamin A supplementation or fortification reduced child mortality. The sole exception was the study conducted in the Sudan, which reported that high-dose vitamin A capsules had no impact on either child mortality or vitamin A deficiency itself *(165)*.

The impact of vitamin A supplementation on morbidity and mortality during measles is striking. Worldwide, measles infection affects approx 30 million children per year, and up to 1 million children may die annually from measles and associated complications. In developing countries, case-fatality rates of 10 to 20% are not uncommon, and a measles episode may result in diarrhea, pneumonia, encephalitis, blindness, and delayed morbidity and mortality after an attack. High-dose vitamin A supplementation to children with acute, complicated measles reduces mortality by approx 50% (Table 3; refs. *157,167,168*). Similarly, the morbidity of diarrhea and pneumonia associated with measles is reduced by vitamin A supplementation *(169,170)*. One study using a lower total dose of vitamin A did not detect any impact of vitamin A supplementation on measles (171). It is notable in these studies that high-dose vitamin A supplementation was shown to reduce morbidity and mortality in children without any clinical signs of vitamin A deficiency.

Vitamin A supplementation has a dramatic impact on the severity of infectious disease morbidity and childhood mortality; however, it seems to have less effect on mild-to-moderate morbidity. Community-based trials of vitamin A supplementation have not demonstrated a consistent effect on the incidence of mild respiratory disease and diarrhea. Morbidity from respiratory infection, fever, and diarrhea (as measured by a history obtained from the mother or father) tend to be subjective and imprecise. Studies that use weekly or even more frequent monitoring of morbidity are unable to address the issue of severe morbidity, because children who are ill usually are treated. Preschool children in developing countries are constantly being exposed to new pathogens and have high rates of respiratory disease and diarrhea in the first years of life. The dramatic reductions in severe morbidity and mortality noted in hospital- and community-based studies of vitamin A may have led to the inflated expectation that vitamin A possibly reduces the incidence of respiratory disease and diarrhea among children. On an immunological basis, there is little evidence that vitamin A prevents infection by a pathogen that is new to the host and thus has a measurable impact on incidence of infection. However, vitamin A-related immune enhancement may result in a vigorous host response, shorter duration of infection, and less complications. This suggestion comes from clinical trials that show that vitamin A supplementation reduces the severity of infectious disease morbidity as well as mortality *(172)*.

Vitamin A supplementation also has been evaluated as disease-targeted therapy for other types of infections. Several clinical trials have been conducted to determine

Table 3
Vitamin A Supplementation Trials for Specific Infections or Populations

Location	*Year*	*Sample size*	*Results*	*Reference*
Vitamin A for Measles				
London	1932	600	58% decrease in mortality during measles	*157*
Tanzania	1987	180	46% decrease in mortality during measles	*167*
South Africa	1990	189	80% decrease in mortality during measles	*168*
South Africa	1991	60	Decrease in morbidity during measles	*169*
Kenya	1994	235	Decrease in morbidity during measles	*170*
Zambia	1996	200	No effect	*171*
Vitamin A and Diarrheal Disease				
Bangladesh	1992	83	No effect on watery diarrhea	*173*
China	1993	172	Decrease in incidence of diarrhea	*174*
Brazil	1994	1,240	Decrease in severity of diarrhea	*175*
India	1994	174	Decrease in duration of diarrhea	*176*
India	1995	216	Decrease in duration of diarrhea	*177*
Brazil	1996	25	Decrease in duration of diarrhea	*178*
Bangladesh	1998	83	Decrease in morbidity of shigellosis	*179*
Vitamin A for Acute Respiratory Infections				
Guatemala	1995	263	No effect	*180*
Bangladesh	1996	165	Decrease in duration of disease	*181*
Brazil	1997	472	No effect	*182*
Tanzania	1998	687	No effect	*183*
Peru	1998	95	Increase in morbidity	*184*
Vitamin A for Respiratory Syncytial Virus Infection				
Chile	1996	180	No effect	*185*
United States	1996	239	No effect	*186*
United States	1996	32	No effect	*187*
Vitamin A for Tuberculosis				
South Africa	1997	85	No effect	*139*
Vitamin A for HIV Infection				
South Africa	1995	118	Decrease in morbidity in infants	*190*
Tanzania	1997	72	Decrease in mortality	*191*
Tanzania	1998	1075	No effect on birth outcomes	*192*
Uganda	2001	181	Decrease in mortality in preschool children	*193*

(Continued)

Table 3 *(Continued)*

Location	*Year*	*Sample size*	*Results*	*Reference*
Vitamin A Given at Time of Childhood Immunizations				
Indonesia	1995	336	Decrease in seroconversion to measles vaccine, 6 mo	*194*
Indonesia	1997	394	No effect on seroconversion to measles vaccine, 9 mo	*195*
Guinea Bissau	1997	312	No effect on seroconversion to measles vaccine, 9 mo	*196*
Ghana, India and Peru	1998	9424	No effect on morbidity, mortality	*197*
Indonesia	1999	467	No interference with seroconversion to live oral poliovirus vaccine	*76*
India	2002	399	No interference with seroconversion to live oral poliovirus vaccine	*75*
Vitamin A Given at Birth				
Indonesia	1996	2067	Decrease in mortality	*198*
India	1996	909	No effect on morbidity	*200*
India	2003	11,619	22% decrease in mortality	*199*
Vitamin A Supplementation During Pregnancy				
Sheffield	1931	550	Reduction in morbidity of women	*31*
Nepal	1999	19,131	30% decrease in pregnancy-related mortality	*143*
Vitamin A in a Malaria Endemic Area				
Papua New Guinea	1999	484	Decrease in morbidity	*203*

whether vitamin A supplementation reduces the severity and duration of diarrhea *(173–179)*. Generally, these studies showed that high-dose vitamin A supplementation reduced the duration of diarrhea, and there was consensus that vitamin A supplementation reduced diarrheal morbidity. Trials of high-dose vitamin A supplementation suggest that there is little to no effect on the duration or severity of acute lower respiratory infections in children *(180–184)* or on respiratory syncytial virus infection in infants and children *(185–187)*. Analysis of data from the large community-based studies suggests that vitamin A supplementation has no impact on acute lower respiratory infections *(188)*. A small study suggested that high-dose vitamin A supplementation had no impact on pulmonary tuberculosis in children *(189)*. During HIV infection, vitamin A supplementation has been shown to reduce diarrheal morbidity in infants *(190)*, and a stratified analysis from a clinical trial suggested that vitamin A supplementation reduced mortality among HIV-infected children with pneumonia *(191)*. Antenatal supplementation with multivitamins reduced fetal deaths and low birth weight among HIV-infected pregnant women, but no significant effect was noted with vitamin A alone *(192)*. A recent controlled clinical trial showed that periodic high-dose vitamin A supplementation reduced mortality by 46% among HIV-infected children in Uganda *(193)*.

The institution of childhood immunization programs, such as the Expanded Programme on Immunization (EPI), has been suggested for the delivery of vitamin A supplements to infants. It was found that high-dose vitamin A supplementation interfered with seroconversion to standard titer live measles vaccine in 6-mo-old infants who had high titers of maternally acquired antibodies to measles *(194)*; however, subsequent studies showed that high-dose vitamin A supplementation did not interfere with seroconversion in 9-mo-old infants *(195,196)*. A multicenter trial showed that vitamin A supplementation administered through the EPI had no effect on morbidity and mortality *(197)*. Vitamin A supplementation given simultaneously with live trivalent oral poliovirus vaccine did not interfere with seroconversion to any of the three poliovirus types *(75,76)*. The available data suggest that integration of vitamin A supplementation with childhood immunization programs at 6, 10, and 14 wk as well as at 9 mo improves vitamin A status of infants and does not have any deleterious effects on response to immunization.

Vitamin A supplementation at the time of birth reduced infant mortality in Indonesia *(198)*. A controlled clinical trial of 11,619 newborn infants showed that those who received 24,000 IU of vitamin A on days 1 and 3 after delivery had 22% lower mortality than infants who received placebo *(199)*. Another study showed that vitamin A supplementation to mother and infant had no effect on infant morbidity *(200)*. Currently, it is unclear whether vitamin A supplementation has an effect on the morbidity or mortality of infants, especially those infants younger than age 6 mo *(197,201)*.

A large community-based trial in lowland Nepal showed that weekly vitamin A or β-carotene supplementation to women of childbearing age reduced maternal mortality by about 30 to 50% *(202)*. This trial raises the possibility that improving micronutrient status of women during pregnancy may reduce maternal mortality.

A recent trial conducted in Papua, New Guinea demonstrated a novel approach toward the immense morbidity and mortality of *Plasmodium falciparum* malaria. Periodic high-dose vitamin A supplementation in a malaria-endemic region was shown to significantly reduce malaria-related morbidity in preschool children *(203)*. Most efforts directed toward malaria have focused on insecticide-impregnated bednets and malaria vaccines, although vitamin A supplementation—an extremely inexpensive and simple intervention—may have comparable effects at a much lower cost. The presumed mechanism by which vitamin A reduces malaria morbidity is by accelerating the acquisition of immunity to malaria. Further studies of the effects of vitamin A on immunity to malaria are underway.

7.3. Current Indications for Use of Vitamin A Supplements in Developing Countries

High-dose oral vitamin A supplements are recommended for children in three general situations: (a) for the treatment of xerophthalmia, (b) for the treatment of acute measles, and (c) as periodic supplementation for the prevention of vitamin A deficiency *(204,205)*. For the treatment of xerophthalmia or acute measles, 200,000 IU (the equivalent of 60 mg of retinol) of vitamin A is administered upon diagnosis, the following day, and 1 mo after for children over age 1 yr, adolescents, and adults (except women of reproductive age). For children under age 1 yr or children of any age who weigh less than 8 kg, the aforementioned guidelines are followed using 100,000 IU (the equivalent of 30 mg of retinol)-capsules. Many developing countries have instituted periodic high-dose vitamin A capsule distribution, and these programs generally use 200,000 IU capsules every 3 to 6 mo for children ages 1 to 6 yr.

For women of reproductive age, pregnant or nonpregnant, the WHO/UNICEF/International Vitamin A Consultative Group recommendations are a daily dose of 10,000 IU of vitamin A for 2 wk. Although this is a general recommendation, this dose often is unavailable in many developing countries. The daily value for vitamin A in antenatal vitamins in the United States is 8,000 IU/d, and many commercial vitamins contain this dose in the form of vitamin A, β-carotene, or a combination of both. In general, high-dose vitamin A capsules should not be used for pregnant women or women of reproductive age who are not using reliable contraception because of concerns regarding the theoretical teratogenicity of vitamin A. It is also important to recognize that there are well-defined therapeutic indications in which vitamin A has been demonstrated to be efficacious, and indiscriminate use of vitamin A supplements should be discouraged.

7.4. Other Studies of Vitamin A in Infants

A large, multicenter, controlled clinical trial was conducted to determine whether vitamin A (5000 IU administered intramuscularly three times a week) could reduce mortality and chronic lung disease among 807 extremely low birth-weight infants who needed respiratory support after birth *(206)*. Infants who received vitamin A had reduced risk of death or chronic lung disease at 36 wk postmenstrual age (RR = 0.89, 95% CI 0.80–0.99) *(206)*. The mean birth weights in the vitamin A and control groups were 770 and 769 g, respectively. A subsequent, relatively small trial involving 154 preterm infants (birth weight: <1000 g) showed that 5000 IU of vitamin A given orally did not reduce the incidence of chronic lung disease after 28 d *(207)*. Meta-analysis of vitamin A trials for very low birth-weight infants showed that vitamin A supplementation resulted in benefits in reducing death or oxygen requirement at age 1 mo *(208)*. Recently, a case–control study showed an association between lack of vitamin A supplementation and sudden infant death syndrome (SIDS) *(209)*. This study suggested a possible link between vitamin A deficiency and SIDS, although further studies are needed to confirm this relationship.

8. PREVENTION OF VITAMIN A DEFICIENCY

8.1. Nutrition Education

The richest dietary sources of vitamin A are liver, egg yolk, butter, cheese, and whole milk, and in most developing country settings, these foods tend to be relatively expensive. Provitamin A carotenoids are found in dark green leafy vegetables, carrots, and citrus fruits such as mango and papaya; however, the bioavailability of these provitamin A carotenoids may be lower than previously thought *(210–213)*. Many efforts have been made to improve knowledge and attitudes regarding food sources that are rich in vitamin A, but it may be difficult for poor families in developing countries to increase consumption of vitamin A-rich foods, especially from animal food sources.

8.2. Food Fortification

Vitamin A deficiency was relatively common in the United States and Europe in the early part of the 20th century, and efforts to improve vitamin A intake included fortification of centrally distributed food products with vitamin A. In 1939, the Council on

Foods and Nutrition of the American Medical Association (AMA) recommended that margarine be fortified with vitamin A *(214)*. In the early 1950s, skim milk (which lacked vitamin A) was fortified with 2000 IU of vitamin A per quart; in 1961, the Food and Nutrition Board of the US National Nutrition Council and the Council on Foods and Nutrition of the AMA reaffirmed their endorsement that margarine, fluid skim milk, and dry nonfat milk be fortified with vitamin A *(215)*.

In some developing countries, such as Guatemala, fortification of sugar with vitamin A has been successful in reducing vitamin A deficiency *(216)*. Vitamin A-fortified margarine improved the vitamin A status of preschool children in the Philippines *(217)*. Similarly, fortification of wheat flour with vitamin A improved vitamin A status of school children *(218)*. Other mechanisms for fortification with vitamin A include instant noodles, edible oils, and monosodium glutamate. Fortified foods appear to make an important contribution toward vitamin A intake in young children. Among poor families in Guatemala, fortified foods contributed half of the recommended vitamin A intake *(219)*. The low prevalence of vitamin A deficiency among preschool children in the Cook Islands has been attributed partially to the requirement that certain imported foodstuffs be fortified *(220)*. Fortified foods may help to overcome insufficient dietary intake of vitamin A in populations where consumption of meat and dairy products is low.

8.3. Homestead Food Production

Homestead food production includes the production of animal foods (such as poultry, small animal production, and fish ponds) and the practice of homestead gardening to grow food for the family and to provide additional income *(221)*. In the current biomedical model for nutrition and health in developing countries, local and family level initiatives often have been ignored in favor of vertical health programs. Homestead food production is an attractive strategy that may be sustainable and may allow empowerment of poor families and communities. The health and nutritional status of the lowest income families usually is the poorest; therefore, homestead food production may have the greatest benefit where such an impact is needed *(222)*. In many developing countries worldwide, vitamin A, iron, and zinc deficiencies are considered a public health problem among women and preschool children and are associated with increased infectious disease morbidity, maternal and child anemia, and child growth retardation.

Although homestead food production has been advocated as a strategy to improve vitamin A status, there are limited data demonstrating an impact of this strategy on micronutrient status and health *(223,224)*. Homestead food production may be a basic factor that increases food availability and intrahousehold food distribution and improves household economic status, with an immediate effect on nutritional status and infectious disease morbidity *(224)*. A recent study from South Africa suggests that home gardening may improve vitamin A status in preschool children *(225)*.

8.4. Other Strategies

The gene for β-carotene synthesis in *Narcissus pseudonarcissus*, a daffodil, has been incorporated into the rice genome, yielding golden rice *(226,227)*. This β-carotene-rich variety of rice has the potential to reduce vitamin A deficiency in Southeast Asia—the part of the world where the prevalence of vitamin A deficiency has been high among children. The safety and efficacy of golden rice in improving vitamin A status of preschool children have not yet been characterized. Potential hurdles in the implementation of this

promising strategy include special interest groups that are opposed to any form of genetically engineered crop. Treatment for intestinal parasites such as *Ascaris lumbricoides* may help to improve the vitamin A status of children who consume a diet high in provitamin A carotenoids *(228,229)*.

9. SUMMARY AND RECOMMENDATIONS

Vitamin A is a potent immune enhancer that has shown efficacy in reducing morbidity and mortality from some infectious diseases among children in developing countries and has reduced blindness among children worldwide. Improved vitamin A nutriture is expected to prevent 1.3 to 2.5 million deaths worldwide each year, and periodic high-dose vitamin A supplementation is now widely practiced in many developing countries where vitamin A deficiency is endemic. High-dose vitamin A supplementation for children with acute, complicated measles may also contribute to a reduction in child mortality, given the estimated 1 million deaths from measles each year, and may also contribute to a reduction in measles blindness *(96)*. A variety of approaches, such as food fortification, supplementation, nutrition education, and home gardening may be used in developing countries to combat vitamin A deficiency and increase resistance to infectious diseases.

As a disease-targeted therapy, vitamin A supplementation has been shown to enhance immunity and reduce the morbidity of measles and diarrheal disease, and there are other potential applications for vitamin A, such as adjunct therapy for tuberculosis. A recent clinical trial suggests that enhancement of immunity to malaria using high-dose vitamin A may be a potential strategy to reduce morbidity and mortality by *P. falciparum* malaria. In situations where daily supplements could be delivered, the use of multivitamins including vitamin A is being evaluated for improvement of birth outcomes and reduction of maternal mortality.

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VII Optimal Pregnancy/Infancy Outcomes

24 Folic Acid-Containing Multivitamins and Primary Prevention of Birth Defects

Andrew E. Czeizel

KEY POINTS

- Neural-tube defects are preventable by periconceptional folic acid or multivitamin supplementation.
- The incidence of some other structural birth defects also can be reduced by folic acid-containing multivitamin use during the periconception period.
- All women of childbearing age who are capable of becoming pregnant should consume folic acid and/or folic acid-containing multivitamins during the periconception period.
- The primary prevention of birth defects by periconceptional folic acid/multivitamin supplementation is much better than the so-called secondary prevention (i.e., the termination of pregnancy because of severe fetal defects).
- In addition to other benefits, periconceptional care is optimal for the introduction of periconceptional folic acid/multivitamin supplementation.
- Food (flour) fortification is the most practical means of supplementation of folic acid and other vitamins for all women.
- Proper preparation for conception is the earliest and most effective method for the prevention of birth defects.

1. INTRODUCTION

The deficiency or overdosage of certain nutrients has a possible role in the origin of birth defects. In 1932, Hale *(1)* demonstrated that a vitamin A-free diet during early pregnancy in sows resulted in offspring that lacked eyes. Some of the pigs also had other defects such as oral clefts, accessory ears, malposition of kidney, and defects of hind legs. Hale's concluded, "The condition is illustrative of the marked effect that a deficiency may have in the disturbance of the internal factors that control the mechanism of development." Further development of experimental teratology became possible when small rodents were introduced for this purpose. Joseph Warkany, one of the founders of

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teratology, recognized the importance of purified diets and used these to test various vitamin deficiencies for their teratogenic effects. Warkany *(2,3)* found that maternal dietary deficiency can induce structural birth defects, that is, congenital abnormalities. Marjorie Nelson *(4)* introduced the use of antimetabolites, which enabled conversion of long-term nutritional experiments into short-term chemical testing. First, antimetabolites of folic acid were used and folic acid deficiency was proved highly teratogenic in pregnant rats *(5–7)*, producing multiple congential abnormalities, neural tube defects (NTDs), orofacial clefts (OFCs), and other congenital abnormalities. This finding was later confirmed in man *(8–10)* as well. This research approach also had strong support from the French investigator Giroud *(11,12)*. In 1964, Hibbard *(13)* reported a higher rate of congenital abnormalitiess (3%) in the infants of folate-deficient mothers than in controls (1.6%); Hibbard and Smithells *(14)* later showed a relationship between human embryopathies and a deficiency of folate metabolism. Therefore, these findings highlighted the developmental importance of folate and/or folic acid. However, this first phase of history of malnutritional teratology including folate deficiency was followed by a longer, silent period.

The second phase of history regarding malnutritional teratology is related to the intervention studies to check the efficacy of folic acid or folic acid-containing multivitamin use for the primary prevention of recurrent NTDs and cleft lip ± palate in the 1980s.

Anencephaly and spina bifida (aperta or cystica) are the major classes of NTDs. Although the genetic (polygenic) background of nonsyndromic NTDs (the so-called isolated NTDs; 92% of all cases) obviously results from the fact that recurrence in first-degree relatives is 10 times greater than their occurrence *(15)*, this group of congenital abnormalities is very sensitive to environmental factors. The latter is indicated by the very wide range (0.5–12/1000) of NTD incidences in different populations, rapid secular changes in their occurrence, seasonal variation of births with NTD, and, mainly, by their obvious socioeconomic status dependence (the risk of NTDs was found to increase from a low risk in the highest class to an above-average risk in the lowest class in the United Kingdom and some other countries *[16]*).

OFCs are also differentiated as isolated (nonsyndromic) or syndromic (multiple) groups. The most frequent types of isolated OFCs are cleft lip ± palate (about 1/1000) and posterior cleft palate (about 0.5/1000). Both types of OFCs are caused by polygene–environmental interaction *(17)*.

2. INTERVENTION STUDIES FOR REDUCTION OF RECURRENT NTDS AND OFCS

The documented start of the second phase of folic acid history occurred in 1976. Smithells *(18)* hypothesized that among triggering environmental factors in the origin of NTDs, undernutrition could be the common denominator, and his group tested this hypothesis: a lower concentration of red cell folate, riboflavin, and vitamin C was found during the first trimester of pregnancy in women who later gave birth to an infant with an NTD compared to matched controls. These findings prompted Smithells and his group to organize the first intervention study to test the effect of diet supplemented with folic acid and some other vitamins *(19)*. Generally, NTDs occur between postconceptional days 15 and 28 in humans; most women are unaware of their pregnancy at this "critical period" of NTDs. Thus, this supplementation should commence at least 28 d

prior to conception and continue to the date of the second missed menstrual period (i.e., during the periconceptional period).

The final results of the Smithells study were published separately for the Yorkshire region of the United Kingdom *(20)* and Northern Ireland *(21)*. However, the 91 and 83% reductions in NTD recurrence (Table 1), respectively, were not accepted by some experts because of possible selection bias. Two ethical committees refused to give permission for the original protocol of a randomized controlled trial; therefore, the control group was made up of women who had already given birth to one or more infants with NTDs and were already pregnant when referred to the study centers or of women who declined to take part in the intervention portion of the trial.

The study performed by Laurence et al. *(22)* in Wales was a randomized, double-blind trial; however, the difference between the number of NTDs in the active and placebo groups was not significant because of the small number of women.

Thus, in the early 1980s, the Medical Research Council (MRC) in the United Kingdom decided to organize a multicenter (43% of participants came from Hungary), randomized, double-blind controlled trial (RCT) (Table 1; ref. *23*). The MRC Vitamin Study found that a pharmacological dose (4 mg) of folic acid supplementation alone reduced NTD recurrence significantly by 71% (relative risk [RR] = 0.29, 95% confidence interval [CI] 0.12–0.71). There was some reduction in the recurrent NTD after the use of other vitamins (2.6%), but it did not differ significantly from the unsupplemented group (4.3%). Based on these results, the Centers for Disease Control (CDC) *(24)* recommended daily supplementation with 4 mg of folic acid under medical supervision in the periconception period for women at high risk (i.e., those who previously gave birth to one or more offspring with NTD) for the reduction of NTD recurrence. The RCT of the Irish Vitamin Study Group was interrupted *(25)*.

In 1982, Tolarova *(26)* reported a protective effect of multivitamins and folic acid for the recurrence of cleft lips . She used a very high dose (10 mg) of folic acid during the periconceptional period.

There were two major questions following the publications of the above "recurrence" studies. First, does folic acid or folic acid-containing multivitamin supplementation also reduce the risk of first occurrence of NTD and/or OFC? About 95% of women who deliver infants with NTD or OFC have no previous NTD or OFC pregnancies; therefore, the prevention of first occurrence of these congenital abnormalities would be a significant public health success. The second question regarded the dose. The pharmacological dose (e.g., 4 mg) of folic acid may have some adverse effects, and, thus, it cannot be recommended for the population at large and/or without medical supervision. The Hungarian randomized, double-blind controlled trial attempted to provide data to answer these questions. The Hungarian RCT was based on the periconception service, thus it is worth summarizing the goals and steps of this new medical service.

3. PERICONCEPTION CARE: PLANNING FOR PREGNANCY FOR BOTH PARTNERS

The provision of preconception care was included in the National Health Promotion and Disease Prevention Objectives for 2000 in the United States *(27)*. We prefer to use the term periconception rather than preconception because prenatal care usually begins in about weeks 8–12 of pregnancy; thus, the early postconceptional period would be

Table 1
The Data From Intervention Studies for the Reduction of NTD Recurrences

Method	*Country*	*Supplement*	*With supplement*		*Without supplement*		*Risk reduction (%)*
			No.	*%*	*No.*	*%*	
Nonrandomized	Yorkshire *(20)*	Multivitamin[a]	1/187	0.5	18/320	5.6	91
	Northern Ireland *(21)*		4/511	0.8	17/353	4.8	83
Randomized	Wales *(22)*	Folic acid (4 mg)	2/60	3.3	4/51	7.8	58
	Multicenter MRC *(23)*	Folic acid (4 mg)	2/298	0.7	13/300	4.3	84
		Folic acid + other vitamins	4/295	1.4	13/300	4.3	67
		Together	6/593	1.0	21/602[b]	3.5	71
		Other vitamins	8/302	2.6	13/300	4.3	40
	Irish Vitamin Study *(25)*	Folic acid (0.4 mg)	0/172	0.0	1/85	1.2	–

[a]Included 0.36 mg of folic acid.
[b]Including cases supplemented with "other" vitamins.

omitted from medical health service in the United States. Therefore, during their most sensitive and vulnerable early period (from the third postconceptional week until the eighth week), fetuses are uncared for and generally unprotected.

The Hungarian Periconception Service (HPS) *(28)* begins 3 mo before pregnancy and continues for 3 mo in the postconceptional period. The HPS was launched in 1984 and includes information counseling, examinations, and interventions performed or supervised by qualified nurses. The main goal is to provide information and care for prospective parents to protect the health of the mother; reduce untoward pregnancy outcomes, birth defects, and developmental disabilities; and increase the potential for infants to be born as healthy as possible.

The HPS consists of three steps (Table 2):

1. Check-up of reproductive health: A medical check-up as a "preconceptional screening" is an essential component for risk identification and assessment and referral of couples or persons to appropriate secondary care. This time and occasion also is optimal for predictive genetic testing of prospective parents followed by risk estimation for their children's future lives. We have made an extra effort to incorporate males into the HPS to help in the development of responsible fatherhood, and it appears to be successful: 86% of females were accompanied by their husbands or partners.
2. The 3-mo preparation for conception: The preparation for conception is an appropriate period to stop smoking, drinking alcohol, and taking unnecessary drugs with hazards to germ cells and later the fetus. This 3-mo preparation period is an optimal time for the launch of periconceptional folic acid or multivitamin supplementation. All women were supplied with a folic acid-containing multivitamin (Elevit pronatal®), free of charge.
3. The better protection of early pregnancy: In the past, pregnancies were diagnosed after the second missed menstrual period; therefore, the most sensitive period of early fetal development was not recognized and protected. In the HPS, after the 3-mo preparation for conception, females are asked to continue the intake of the multivitamin, to achieve conception on the optimal day (1 d prior to ovulation), and to visit the HPS immediately after the first missed menstrual period. The goal of this visit is pregnancy confirmation by a sensitive pregnancy test. At that time, an ultrasound scanning is suggested for 2 wk later (nearly all ectopic pregnancies were diagnosed as a result of this examination, such an early phase assessment allowed for conservative treatment instead of surgery). Women with known occupational hazards were exempted and informed regarding how to avoid other risks (e.g., heat bathing, exposure to infected cats), and pregnant women were asked to continue multivitamin supplementation and were referred to the prenatal care clinic with the discharge summary of the HPS.

The periconception care is appropriate for the detection of infertile couples and to treat them sooner; to provide a more effective identification of couples with positive family history, case history, and/or sexually transmitted infections/disorders; and to provide the appropriate secondary care. The HPS is optimal for the introduction of periconceptional multivitamin/folic acid supplementation and predictive genetic testing, for improvement in the diet, and for cessation of smoking and alcohol consumption before conception. Obviously, proper preparation for conception is the earliest and probably the most important method of health promotion in general and for the prevention of birth defects in particular.

Table 2
Three Steps and Different Items of the HPS

1. Check-up of reproductive health
    a. Family history of females and males.
    b. Case history of females (e.g., epilepsy, diabetes, etc.)
    c. Vaginal and cervical smear examination for the screening of sexually transmitted infections/disorders.
    d. Sperm analysis for the detection of subfertility and pyosperm.
    e. Psychosexual exploration of couples.
    f. Blood examination for the revealing of rubella seronegative women (they are vaccinated), anemia and HIV positivity, in addition recently for predictive gene diagnostic tests.
2. The 3-mo preparation for conception
    a. Necessary further examinations and/or treatments in the disorders of couples detected at the check-up examination.
    b. Protection of germ cells: avoidance of smoking, alcohol, and unnecessary drugs.
    c. Discontinuation of contraceptive pills to restore the internal hormonal balance of females (condoms are provided).
    d. Occupational history to reveal occupational hazards (to contact occupational doctor).
    e. Measurement of basal body temperature for detection of hormonal dysfunction (and treatment, if necessary) and determination of the optimal day of conception.
    f. Preconceptional multivitamin supplementation.
    g. Suggestion to check dental status.
    h. Guidelines for physical exercise.
    i. Guidelines for healthy diet.
3. The better protection of early pregnancy
    a. To achieve conception on the optimal day.
    b. Early pregnancy confirmation.
    c. Periconceptional multivitamin supplementation.
    d. Avoidance of teratogenic and other risks.
    e. Referral of pregnant women to prenatal care clinics.

HPS, Hungarian Periconceptional Service; HIV, human immunodeficiency virus.

4. THE HUNGARIAN RANDOMIZED CONTROLLED TRIAL

The Hungarian RCT was performed in the coordinating center of the HPS in my previous department. The goal was to test the preventive effect of a physiological dose (0.8 mg) of folic acid as one component of a multivitamin (Elevit prenatal) used during the periconceptional period for the first occurrence of NTD and OFC. The multivitamin contained 12 vitamins, including folic acid (0.8 mg), vitamin B_{12} (4.0 µg), B_6 (2.6 mg), B_2 (1.8 mg), C (100.0 mg), four minerals, and three trace elements. The use of true placebo was not allowed by the ethical committee, thus a placebo-like trace element combination (including the three trace element components: copper, manganese, and zinc of Elevit prenatal) was used. The Hungarian RCT was launched on February 1, 1984, and the recruitment was closed on April 30, 1991; pregnancy outcomes were evaluated until the end of April 1993.

Table 3
First Occurrence of NTDs in Informative Offspring of Study Groups in the Two Hungarian Intervention Studies

Intervention trials	*Supplement*		*No supplement*
Randomized controlled trial			
No. of offspring	2471		2391
Expected/observed number of NTD	6.9/0		6.7/6[a]
RR (with 95% CI)		0.07 (0.04–0.13)	
Two-cohort controlled study			
No. of offspring	3056		3056
Expected/observed number of NTDs	8.5/1[b]		8.5/9[c]
OR (with 95% CI)		0.11 (0.01–0.91)	
Pooled data			
No. of offspring	5527		5447
Expected/observed number of NTDs	15.4/1		15.2/15
OR (with 95% CI)		0.08 (0.01–0.47)	

[a]Anencephaly 2. anencephaly + spina bifida (lumbal, thoracolumbal) 2. Spina bifida (thoracolumbal, lumbosacral) 2.
[b]Anencephaly 1.
[c]Anencephaly 1, Spina bifida 8 (thoracolumbal 1, lumbal 4, lumbosacral 3).
NTDs, neural tube defects; RR, relative risk; CI, confidence interval; OR, odds ratio.

As demonstrated in Table 3, no NTD case was found among informative offspring (liveborn infants until age 1 yr, stillborn fetuses, and prenatally diagnosed malformed fetuses after the elective termination of pregnancy) in the multivitamin group, whereas six informative offspring with NTD occurred in the placebo-like trace element, ($p = 0.01$). Therefore, the Hungarian RCT demonstrated that a multivitamin containing 0.8 mg of folic acid prevented about 90% of the first occurrences of NTD *(29)*. However, the reduction of OFC was not confirmed after the use of the folic acid-containing multivitamin (Table 4).

Based on the Hungarian RCT and some observational studies, in September 1992, the CDC *(30)* recommended that "all women of childbearing age who are capable of becoming pregnant should consume 0.4 mg of folic acid per day for the purpose of reducing their risk of having a pregnancy affected with spina bifida or other neural tube defects," and this recommendation was subsequently followed by several countries.

The great majority of the women in the Hungarian RCT were healthy and not malnourished *(31)*; therefore, this trial was appropriate to study several other effects of periconceptional folic acid-containing multivitamin supplementation.

During the preconceptional multivitamin supplementation, the female cycle became more regular, that is, the variance was lower *(32)*. Thus, multivitamin supplementation may have a beneficial effect for women with irregular menstrual cycles.

There was no difference in the sexual activity (measured by the rate of weekly sexual intercourse) of couples between the multivitamin and the no multivitamin groups in the preconceptional period *(33)*. However, only women were supplemented, and sexual activity often is determined by males.

Table 4
The Efficacy of Periconceptional Folic Acid-Containing Multivitamin (Micronutrient) Supplementation in the Primary Prevention of Some Major Congenital Abnormalities Groups

	Hungarian intervention trials					
	RCT		*TCS*		*Pooled data*	
Congenital abnormalities groups	*No supplement* (n = 2391)	*Supplement* (n = 2471)	*No supplement* (n = 3056)	*supplement* (n = 3056)	*No Supplement* (n = 5447)	*Supplement* (n = 5527)
Urinary tract congenital abnormalities						
Renal a/dysgenesis	3	0	0	2	3	2
Cystic kidney	1	1	0	2	1	3
Obstructive congenital abnormalities						
Pelvic ureteric junction	4	0	13	2	17	2
Other locations	1	1	6	8	7	9
Total	9	2	19	14	28	16
OR (95% CI)	0.21 (0.05–0.95)		0.71 (0.33–1.50)		0.56 (0.30–1.04)	
Cardiovascular congenital abnormalities						
Conotruncal						
Ventricular septal defect	8	2	19	5	27	7
Others	2	1	1	3	3	4
Subtotal	10	3	20	8	30	11
Others	10	7	30	23	40	30
Total	20	10	50	31	70	41
OR (95% CI)	0.42 (0.19–0.98)		0.60 (0.38–0.96)		0.57 (0.39–0.85)	

Congenital limb deficiencies						
Terminal transverse	2	1	3	1	5	2
Others	3	0	0	0	3	0
Total	5	1	3	1	8	2
OR (95% CI)	0.19 (0.03–1.18)		0.33 (0.01–3.71)		0.25 (0.05–1.16)	
Congenital pyloric stenosis	8	2	2	0	10	2
OR (95% CI)	0.24 (0.05–1.14)		0.00 (0.00–26.8)		0.20 (0.04–0.90)	
Anal/rectal atresia/stenosis	1	0	4	1	5	1
OR (95% CI)	—		0.31 (0.02–2.52)		0.20 (0.02–1.69)	
Orofacial clefts						
Cleft lip ± palate	3	4	2	3	5	7
Posterior cleft palate	2	0	1	1	3	1
Total	5	4	3	4	8	8
OR (95% CI)	0.77 (0.22–2.69)		1.63 (0.31–2.88)		0.99 (0.37–2.63)	
Down syndrome	5	2	8	8	13	10
OR (95% CI)	0.39 (0.07–1.99)		0.76 (0.33–1.73)		0.76 (0.33–1.73)	
Unidentified multiple congenital abnormalities	5	6	15	12	20	18
OR (95% CI)	1.16 (0.35–3.81)		0.89 (0.45–1.68)		0.89 (0.47–1.68)	

A 7% higher rate of conceptions occurred in women who were treated with the multivitamin preconceptionally compared with those who were not supplemented. The time taken to become pregnant was slightly but significantly shorter in the multivitamin group *(34)*.

A significantly lower rate of treated morning sickness (nausea and vomiting) occurred in early pregnancy after periconceptional multivitamin supplementation (3 vs 6.6% in the no multivitamin group) *(35)*. There was no difference in maternal weight gain between the multivitamin and no multivitamin groups before and during pregnancy *(36)*.

All possible side effects were monitored continuously. Until January 1999, the number of female participants in the Coordinating Center of the HPS was 14,540, and patients with pernicious anemia were not recorded among these reproductive-aged women. Among female participants, 66 (0.46%) were epileptic, and 60 wanted to use multivitamin supplementation. There was no case with multivitamin-related side effects of epilepsy during the periconception period *(37)*. However, a 22-yr-old woman with epilepsy was treated continuously with carbamazepine and another folic acid-containing multivitamin (1 mg) from the 20th wk of gestation, and she had status epilepticus and later developed symptoms of systemic lupus erythematosus *(37)*. Autoimmune disease of pregnant women with epilepsy could damage the blood–brain barrier, and the pharmacological dose (≥1 mg) of folic acid may trigger a cluster of seizures. Of 14,540 female participants, 4 (0.03%) had severe allergic exanthema; of these, 3 discontinued the use of multivitamin (all had a history of drug-induced allergic diseases). Among all other possible side effects, constipation (1.8 vs 0.8%) and diarrhea (1.4 vs 0.4%) were reported somewhat more often after multivitamin supplementation in the preconceptional period *(36)*.

The rate of multiple births, namely twins, was about 40% higher after periconceptional multivitamin supplementation *(38)*. The higher rate of twin conceptions could not be explained by maternal factors (e.g., age, parity) or by a higher rate of infertility drug use *(39)*. This finding was confirmed later in the United States *(40)* and Sweden *(41)* but not in China *(42)*. However, the prevalence of twins is lowest in the Asian race (including the Chinese population), and Chinese people have an extremely high occurrence (55%) of TT genotype of the *methylenetetrahydrofolate-reductase* (*MTHFR*) gene *(43),* and this genotype associates with a very low dizygotic twin rate *(44)*. There was a somewhat higher prevalence of multiple pregnancies after periconceptional folic acid/multivitamin supplementation in the 38,151 controls of the Hungarian Case-Control Surveillance of Congenital Abnormalities, which took place in 1980–1996 *(45)*.

There was no significant difference in pregnancy outcomes of singletons, including four types of fetal deaths: (a) chemical pregnancies (positive pregnancy test without clinical symptoms of pregnancy later), (b) ectopic pregnancies, (c) miscarriages (including the so-called missed abortions or blighted ova), and (d) late fetal death (stillbirths) and five variables of liveborn infants (gender ratio, birth weight, gestational age, proportion of low birth weight, and preterm birth) *(46)*, between the multivitamin and no multivitamin groups. The rates of all kinds of fetal deaths were somewhat, but not significantly, higher in the multivitamin group, and together the difference reached the 0.05 level of significance between the multivitamin and no multivitamin groups. Additionally, the ratio of boys to girls showed a slight excess of girls in the multivitamin group, whereas the well-known boy predominance was seen in the no multivitamin group. Thus, a small change in the pattern of prenatal selection cannot be excluded. This finding was confirmed in the United States *(47)* but not in China *(48)*. In my opinion, the slightly higher rate of fetal death may be explained

by the higher proportion of multiple conceptions in the multivitamin group *(49)* because these pregnancies have some excess in the occurrence of fetal death.

Postnatal somatic (body weight, body length, head circumference) and mental (measured by three tests) development until ages 1 *(50)* and 6 yr *(51)* did not show any significant difference between the multivitamin and no multivitamin groups. Therefore, the higher rate of worrying, fussiness, and fearfulness found previously in girls born after periconceptional multivitamin supplementation *(52)* was not confirmed.

Finally, there was a significant reduction in the total rate of informative offspring with congenital abnormalities after periconceptional multivitamin supplementation. The rate of major congenital abnormalities was 20.6 and 40.6 per 1000 in the multivitamin and in the no multivitamin groups, respectively (RR = 0.53, 95% CI 0.35–0.70) *(53)*. After the exclusion six NTD cases, the difference in the rate of major congenital abnormalities between the two study groups remained very highly significant ($p < 0.0001$). In conclusion, periconceptional multivitamin supplementation reduced not only the occurrence of NTD but also the rate of other major congenital abnormalities *(54)*.

The final data set of the Hungarian RCT indicated a significant reduction in two congenital abnormalities groups *(55)* beyond NTD (Table 4). Nine cases with congenital abnormalities of the urinary tract were found in the no multivitamin group, and only two were found in the multivitamin group. The difference was most obvious in the obstructive congenital abnormalities of the urinary tract. There were 10 infants with cardiovascular congenital abnormalities in the multivitamin group and 20 in the no multivitamin group. This difference was explained mainly by two cases of ventricular septal defect in the multivitamin group and eight cases in the no multivitamin group. The difference in the rate of conotruncal congenital abnormalities (3 vs 10) also was significant (RR = 0.29 95%CI 0.09–0.97).

There was also some reduction in the prevalence at birth of congenital limb deficiencies and congenital pyloric stenosis; however, the difference did not reach the level of significance between the multivitamin and no multivitamin groups (Table 4).

5. TWO-COHORT CONTROLLED STUDY

For ethical reasons, RCTs could not be continued; therefore, a two-cohort controlled intervention study (TCS) *(56)* was designed to collect more data to confirm or reject the preventive effect of periconceptional folic acid-containing multivitamin supplementation for the reduction of urinary tract and cardiovascular congenital abnormalities, limb deficiencies, and congenital pyloric stenosis. Additionally, we wanted to get a more accurate estimation for the magnitude of NTD prevention and to collect more data regarding OFCs.

Supplemented women were recruited via HPS; however, beyond the coordinating center in Budapest, the TCS included all the 31 countryside centers between May 1, 1993 and April 30, 1996. The examination of cases with congenital abnormalities was performed until April 30, 1999, because of the 1-yr follow-up of infants. These centers supplied all participants with the same folic acid-containing multivitamin (0.8 mg; Elevit pronatal) during the periconceptional period. Final recruitment of supplemented cohort occurred at the 14th wk of gestation. Unsupplemented women were recruited at the 14th wk of pregnancy from the standard regional prenatal care clinics. They were matched to each pregnant woman of the supplemented cohort on the basis of age,

socioeconomic status, and place of residence. Similarly to the RCT, congenital abnormalities were evaluated in the informative offspring of both cohorts based on well-defined diagnostic criteria of congenital abnormalities during three time windows (during pregnancy, at birth, and during the first postnatal year).

A total of 3056 informative offspring pairs were evaluated in the cohort of supplemented and unsupplemented matched mothers. There were 14 and 19 informative offspring with congenital abnormalities of the urinary tract in the supplemented and unsupplemented cohort, respectively. In the group with obstructive congenital abnormalities (10 vs 19), the stenosis of pelvicureteric junction (2 vs 13) showed a significant difference (OR = 0.19, 95% CI 0.04–0.86). The occurrence of cardiovascular congenital abnormalities (31 vs 50) was significantly reduced, explained mainly by the lower occurrence of ventricular septal defect (5 vs 19; OR = 0.26, 95% CI 0.09–0.72) in the supplemented cohort. Limb deficiencies also did not show a real difference between the supplemented and unsupplemented cohort (1 vs 3). However, it is worth mentioning that all cases had terminal transverse type. Congenital pyloric stenosis was diagnosed in two infants of the unsupplemented cohort, whereas this congenital abnormalities did not occur in the supplemented cohort.

The protective effect of folic acid-containing multivitamin for the reduction of NTD was confirmed (one offspring in the supplemented vs nine offspring in the unsupplemented cohort) (Table 3). The occurrence of OFCs (4 vs 3) showed no significant difference between the two cohorts (Table 4).

There was no difference in the rate of multiple congenital abnormalities and Down's syndrome between the supplemented and unsupplemented cohorts (Table 4).

The total number of informative offspring with congenital abnormalities was 41.56 per 1000 in the supplemented cohort and 55.30 per 1000 in the unsupplemented cohort (OR = 0.74, 95% CI 0.38–0.94). The higher total rates are explained by the use of more sensitive ultrasound scanning and the different spectrum of congenital abnormalities.

The comparison of maternal morbidity and previous pregnancy outcomes between the two cohorts showed differences. The cohort of supplemented pregnant women recruited from the HPS had a higher rate of morbidity and considerably more previously unsuccessful pregnancy outcomes (fetal death and congenital abnormalities) compared to the cohort of unsupplemented pregnant women recruited at the prenatal care clinics. This possibly results from the good reputation of the HPS in support of a healthy pregnancy, which might attract women with previous pregnancy problems. However, the results of this cohort study are consistent with the findings of the previous RCT that periconceptional multivitamin supplementation provides a protective effect, beyond NTD, for some cardiovascular congenital abnormalities—mainly ventricular septal defects and obstructive congenital abnormalities of urinary tract, particularly stenosis of pelvicureteric junction. These preventive effects were demonstrated despite the fact that the supplemented pregnant women was a cohort at high risk because of higher maternal morbidity and more unsuccessful previous pregnancy events.

Table 4 shows the pooled number of different congenital abnormalities in the Hungarian RCT and TCS as well, with ORs at 95% CIs.

6. OTHER NTD STUDIES

Another intervention study to check the efficacy of a physiological dose of folic acid for the prevention of first occurrence of NTD was performed in China *(57)*. This study

was part of a public health campaign conducted from 1993 to 1995 that stressed the fact that intake of 0.4 mg of folic acid daily can reduce the risk of NTD (about 79%) in areas with high rates of NTD (3.3–5.5/1000), whereas this reduction was about 16% in areas with low rates (0.8–1.0/1000). However, the NTD risk was reduced by 41% after the periconceptional use of folic acid in the low-rate area.

The results of many observational studies were also published regarding the prevention of NTD in the international literature; however, this review summarizes only the data of intervention trials.

The available epidemiological and biochemical evidence suggests that the origin of NTD is not primarily the lack of sufficient folate in the diet but arises from genetically determined changes in the uptake, metabolism, or both in maternal and, particularly, fetal cells *(58)*. Thus, a gene–environmental interaction between vitamin dependency (e.g., an inborn error of folate metabolism) and nutrition (such as dietary deficiency) may have a causal role in the origin of NTDs. Supplementation with folic acid-containing multivitamins or folic acid alone may cause an increase in folate metabolite concentrations of tissue fluids, and it may overcome the failure of the local folate metabolite supply.

Obviously, some genes and several environmental factors contribute to the origin of NTD. This chapter discusses only hyperhomocysteinemia (the most established hypothesis), because the primary prevention of NTD by folic acid or folic acid-containing multivitamin is partly connected with the reduction of hyperhomocysteinemia. There are several studies that provide evidence of the confirmation of hyperhomocysteinemia-related NTDs.

The causes of hyperhomocysteinemia require a longer explanantion. When meat, fish, or plant proteins are digested, amino acids (e.g., methionine) are released. During the conversion of methionine to cysteine, homocysteine (a toxic metabolite) is formed, and humans neutralize it normally as soon as possible. On one hand, homocysteine can be metabolized via the transsulfuration pathway to form cystathionine caused by the condensation with serine and catalyzed by cystathionine β-synthase. This enzyme requires pyridoxal 5′–phosphate, the biologically active form of vitamin B_6 as a cofactor. On the other hand, remethylation of homocysteine to methionine is catalyzed by methionine-synthase. This enzyme requires vitamin B_{12} as a cofactor and 5-methyltetrahydrofolate as the methyl donor. The latter explains the importance of folate–folic acid deficiency in the origin of NTD.

This vitamin was discovered by Wills *(59)* in 1931, and she stressed the similarity of this "curative agent" with vitamin B_{12} as a "twin" vitamin. The generic term "folate" describes the many different naturally occuring polyglutamate forms of the vitamin, whereas folic acid is the synthesized monoglutamate form, chemically designated as pteroylglutamic acid. The different name of two forms of the same vitamin is disturbing and are confused frequently, thus their joint name as vitamin B_{11} has been used in some countries (e.g., the Netherlands), whereas their name is vitamin B_9 in France.

Humans cannot produce folate. The major dietary sources of folates are fresh and frozen green leafy vegetables, citrus fruits and juices, liver, wheat bread, and legumes. Food folates are converted to monoglutamates by conjugases in the upper part of the small intestine. However, monoglutamate folic acid can be absorbed directly. After the active and passive absorption of monoglutamates, they are converted to dihydrofolate and then to tetrahydrofolate (THF) by reductase enzymes. THF is the parent compound of all biological active folates. The most important cause of hyperhomocysteinemia and/or lack of methionine is the polymorphism of the *MTHFR* gene *(60,61)*. A 677C→T mutation has

been identified in the *MTHFR* gene, and it results in the thermolabile variant (Ala225Val) of *MTHFR (62)* with 30 to 50% activity of the allelic form. This enzyme variant cannot effectively catalize the pathway of 5,10,methylene-THF to 5,methyl-THF (i.e., the methyl donor for methionine-synthase), and it may have a role in the origin of NTD *(63)*. The Hungarian population has a moderate total prevalence of NTD (3/1000), whereas the frequencies of TT and CT genotypes within *MTHFR* genes are 11.1 and 45.2%, respectively *(43)*. The *MTHFR* gene polymorphism is so common in different populations *(43)* that it needs some explanation. The highly significant excess of CT heterozygosity in male first-degree relatives of patients with NTD may demonstrate CT heterozygote advantage in the context of their genetic background *(64)*. Thus, the thermolabile variant of *MTHFR* and the polymorphism of the *MTHFR* gene is an obvious risk factor for NTD. However, other common mutations of the *MTHFR* gene and other genes in folate metabolism may also have a role in the origin of NTD *(65)*. The point is that there is an inverse relation between homocysteine and folate levels in the humans.

Hyperhomocysteinemia and/or lack of methionine can induce NTD in animal experiments *(66,67)*. Mothers who gave birth to a child with NTD have higher blood *(68)* and amniotic fluid *(69)* levels of homocysteine. Low maternal folate status is a risk factor for NTD. Folic acid-containing multivitamins and folic acid alone are able to reduce the hypermomocysteinemia and, consequently, the hyperhomocysteinemia-related NTDs.

The history of NTD shows that we can modify our destiny with the help of science. Before the 1960s, nearly all victims of NTDs had fatal outcomes. In the 1960s, physicians introduced very early complex surgical and medical interventions, and the lives of victims were saved in the majority of spina bifida cases (of course, anencephaly is lethal). In the 1970s, the selective criteria of surgical intervention was introduced to reduce the production of multiple handicapped children. In the 1980s, prenatal screening based on maternal serum α-fetoprotein (MS-AFP) and/or ultrasonography was introduced. In the low-risk populations, the detection rate (i.e., sensitivity) of MS-AFP testing for open NTD varies from 72 to 91% and the specificity varies from 96.2 to 98.7% *(70)*. This prenatal screening resulted in a significant drop in the birth of NTD fetuses; however, it increased the number of pregnancy terminations and fetal death resulting from amniocentesis, which was about 0.5 to 1.0% risk for miscarriage. Finally, since the 1990s, we have had a chance to reduce the maldevelopment of the neural tube because of the intentional modification (supplementation) of the diet, at least in the periconceptional period of the life. Currently, the periconceptional folic acid or folic acid-containing multivitamin supplementation as a primary preventive method of NTD offers an appropriate alternative with the same efficacy of the so-called secondary prevention (i.e., the prevention of the birth birth of babies with NTD). The evaluation of adverse effects regarding primary and secondary prevention shows an obviously better picture for multivitamin supplementation. Very rare adverse effects may occur only after the use of pharmacological doses of folic acid. The cost is much lower in primary compared to secondary prevention. The main moral and medical advantages are the lack of pregnancy termination in primary prevention. Thus, primary prevention obviously is much better than the termination of pregnancy after the prenatal diagnosis of fetal defect.

The periconceptional multivitamin supplementation containing a physiological dose (0.8 mg) of folic acid is very effective in reducing the first occurrence of NTD. About 90% of NTDs seem to be preventable in Hungary using this method (Table 3).

The US–Chinese study had two important messages *(51)*. On one hand, 0.4 mg of folic acid alone was also able to significantly reduce the first occurrence of NTD. On the other hand, the efficacy of prevention by folic acid depended on the incidence of NTD. The data of TCS showed the preventive effect of multivitamin containing 0.8 mg of folic acid for the *recurrence of* NTD as well. In the data set of the TCS, 41 informative offspring had previous siblings or parents with NTD without recurrence in the supplemented cohort. However, the statistical power is limited. Additionally, the data set of our genetic counseling clinic included 93 cases that had siblings or parents with NTD, and there was no recurrent NTD after the periconceptional folic acid-containing multivitamin supplementation. We expected 6.7 recurrent NTDs in these 134 families; fortunately, there was no recurrence. The study of Smithells et al. *(19,20)* also clearly demonstrated the high efficacy (about 90% reduction in the recurrence of NTDs) of a multivitamin containing 0.36 mg of folic acid in the prevention of recurrent NTDs. Finally, the study of Daily et al. *(71)* showed that there was no obvious greater reduction of NTD after the use of higher doses of folic acid.

Several studies indicated the efficacy of folic acid-containing multivitamin supplementation for the prevention of other congenital abnormalities as well, and it is extremely important from the public health aspect because NTDs represent only 3 to 12% of all major congenital abnormalities.

7. HISTORICAL PERSPECTIVE

Presently, 20 to 25% of infant mortality in industrialized countries is caused by congenital abnormalities. However, congenital abnormalities are among the leading causes of death, with a high number of life years lost and impaired life *(72)*. Another important feature of congenital abnormalities is that they represent a defect condition, because it is difficult to achieve a complete recovery. Therefore, prevention is considered the only optimal solution.

Periconceptional use of a multivitamin with less than 1 mg of folic acid did not result in a reduction of *OFCs* in the Hungarian RCT and TCS (Table 4). However, a significant reduction was demonstrated in the prevalence of OFCs at birth in the data set of the Hungarian Case–Control Surveillance of Congenital Abnormalities after the use of a high dose (generally 6 mg) of folic acid alone *(73)*. Hungarian obstetricians generally recommend 6 mg of folic acid for all women in the early phase of pregnancies after the first visit in the prenatal care. Among 22,843 cases with congenital abnormalities and 38,151 matched controls without congenital abnormalities, we were able to identify some women with periconceptional or early postconceptional use of a high dose of folic acid. Thus, a dose-dependent preventive effect of folic acid for OFCs cannot be excluded. Tolarova *(26)* used 10 mg of folic acid in her study. The results of observational studies are controversial *(74,75)*, because beyond the dose, the efficacy of this primary preventive methods depends on the genetic background of population, socioeconomic status, and lifestyle (particularly the diet) of mothers *(76)*.

Cardiovascular congenital abnormalities were reduced in infants born to supplemented mothers both in RCT and TCS (Table 4). The diagnostic criteria and method of "blind" cardiological examination were similar between the RCT and TCS. Ventricular septal defect had an even statistically significant lower rate after periconceptional multivitamin use. Our findings regarding the protective effect of folic acid-containing multivitamins for

cardiovascular congenital abnormalities—mainly conotruncal defects, including ventricular septal defects—were confirmed by three US studies *(77–79)*. These results as well as ours suggest that periconceptional use of multivitamins was associated with about a 40% reduction in risk for cardiovascular congenital abnormalities. However, one US study did not show this protective effect for cardiovascular congenital abnormalities *(80)*. The protective effect of early postconceptional supplementation of a pharmacological dose (6 mg) of folic acid for cardiovascular congenital abnormalities was also seen in the data set of the Hungarian Case–Control Surveillance of Congenital Abnormalities *(81)*. Cardiovascular congenital abnormalities were induced by pteroyglutamic acid deficiency during gestation in rat fetuses *(82,83)*.

The occurrence of obstructive congenital abnormalities of the urinary tract, more specifically the stenosis of pelvicureteric junction, was reduced in newborn infants born to mothers with multivitamin supplementation in both the RCT and TCS (Table 4). In the 1950s, Monie et al. *(84,85)* produced congenital abnormalities of the urinary tract in rat embryos by folic acid deficiency. In human studies, Li et al. *(86)* and Werler et al. *(80)* also found a significant reduction in the rate of urinary tract defects after multivitamin use in the first trimester of pregnancy.

There was some reduction of cases with congenital limb deficiencies, particularly unimelic terminal transverse type, after multivitamin supplementation both in the RCT and TCS (Table 4). Three US studies showed a significant reduction of congenital limb deficiencies after multivitamin supplementation *(77,87,80)*. The teratogenic effect of folic acid deficiency resulting from folic acid antagonists such as aminopterin and methotrexate caused, among other congenital abnormalities, limb deficiencies in human embryos *(8–10)*.

There was a lower rate of cases with congenital pyloric stenosis after multivitamin supplementation both in the RCT and TCS (Table 4); however, the numbers were limited. This finding was not confirmed in a US study *(80)*.

In the China–US Collaborative Project for Neural Tube Defect Prevention *(57)*, a somewhat lower occurrence of rectal atresia/stenosis was found after periconceptional folic acid supplementation *(88)*. The Hungarian RCT and TCS showed a similar trend (1 vs 5) (Table 4).

There was no difference in the rate of multiple congenital abnormalities between the supplemented and unsupplemented groups in the Hungarian RCT and TCS (Table 4); therefore, our data do not confirm the finding of Shaw et al. *(89)* regarding a higher occurrence of multiple congenital abnormalities after periconceptional intake of multivitamin supplements *(90)*. The rate of Down syndrome also was not lower in the informative offspring of supplemented women in the Hungarian RCT and TCS (Table 4), although recent publications showed an association between polymorphism in genes involved in folate metabolism and maternal risk for Down syndrome *(91–93)*. However, the high dose of folic acid indicated some preventive effect for Down syndrome in the Hungarian Case–Control Surveillance of Congenital Abnormalities *(94)*.

Obviously, these findings could have major implications in the general use of periconceptional folic acid-containing multivitamin supplementation to prevent congenital abnormalities. It is strange that although the data concerning the prevention of NTD were accepted with enthusiasm in the international scientific literature in the early 1990s and prompted new recommendations for their practical implementation, the new data regarding the prevention of other congenital abnormalities have been received with

some reservation and without any further recommendations. Recent studies of antifolate drugs have also confirmed their role not only in the origin of NTD *(95)* but in the origin of cardiovascular and urinary tract congenital abnormalities and limb deficiencies *(96,97)*. However, different congenital abnormalities have different etiologies; therefore, it is necessary to clearly identify the association of periconceptional folic acid/folic acid-containing multivitamin use with the specific reduction of the above congenital abnormalities.

The second phase of malnutrition teratology provided scientific evidence of the primary prevention of NTD and other congenital abnormalities by periconceptional multivitamin or folic acid supplementation. However, it is worth stressing that the total reduction in congenital abnormalities without NTD cases was 19.93 per 1000 in the RCT, which exceeded the total prevalence of NTD (2.78/1000) by 7.2-fold in Hungary. This reduction resulted mainly from the partial prevention of cardiovascular congenital abnormalities and the prevalence at birth of the latter congenital abnormalities group in 3.5 times higher than the total prevalence of NTD. Additionally, the so-called secondary prevention of cardiovascular congenital abnormalities is more limited than that of NTD. Thus, the prevention of other congenital abnormalities by this new primary preventive method has a similar significant public health importance to the prevention of NTDs.

In conclusion, folic acid or folic acid-contaning multivitamin supplementation offers a breakthrough in the primary prevention of NTDs and some other congenital abnormalities, because their use in the periconceptional period can reduce about one-third of congenital abnormalities and provide a better alternative than secondary prevention (i.e., the termination of pregnancy after the diagnosis of severe fetal defect).

8. RECOMMENDATIONS FOR NUTRITIONAL INTERVENTION TO REDUCE BIRTH DEFECT RISKS

The primary prevention of NTDs and other congenital abnormalities by dietary supplements represent the third phase of malnutritional teratology; this public health action is a milestone in the history of nutritional science and preventive nutrition. Presently, there are three possibilities to provide appropriate folic acid and other B vitamin intake for women of childbearing age who are capable of becoming pregnant.

8.1. Consumption of Folate-Rich and Other Vitamin-Rich Diets

The preconceptional period is an appropriate time to change the dietary habit and to improve the lifestyle of prospective parents, particularly mothers because of their good compliance resulting from their desires to have a healthy baby. Thus, it is an important task to advise all women to have a diet rich in folate and other vitamins from the preconceptional time onward.

There is evidence that appropriate nutritional status of pregnant women can promote the postnatal—including adult health—of offspring. The origin of many common complex diseases (hypertension, coronary heart disease, diabetes mellitus, obesity) may be related to the quality of fetal and infant life *(98)*. Appropriately preparing parents for pregnancy can also help to educate children at the earliest point (i.e., birth) of life regarding a healthy diet and lifestyle, and there is a good chance that these habits will be fixed later in life.

A study by McPartlin et al. *(99)* suggested that the optimal daily intake of folate/folic acid in the pre- and postconceptional period is about 0.66 mg. This quantity is much higher than the recent Recommended Daily Allowance (RDA), because there is an increased requirement for folate during pregnancy as a result of (a) decreased absorption, (b) accelerated breakdown of folate to *p*-aminobenzoylglutamate and its acetylated derivative *p*-acetamidobenzoylglutamate, (c) increased urinary loss, and (d) fetal transfer. The calculated total fetal and placental THF content is 0.8 mg/100 g at term *(100)*; therefore, fetal blood has a higher THF level than maternal blood and is indicative of active placental transfer *(101)*.

However, the usual daily intake of folate is about 0.18–0.20 mg *(31)*, and it is difficult to imagine a 3.3- to 3.7-fold increase in folate intake every day in anticipation of conception, which would require about 15 servings of broccoli or Brussel's sprouts. Additionally, an extreme increase in the consumption of extra folate from natural food is relatively ineffective at increasing folate status *(102)*.

Three other B vitamins play important roles in folate and/or homocysteine metabolism. The methyl group of 5-methyl-THF is used by methionine synthase to recycle homocysteine back to methionine, and methionine synthase is a vitamin B_{12}-dependent enzyme. Vitamin B_{11} is important for DNA and RNA biosynthesis as well. These functions may explain why vitamin B_{11} deficiency is an independent factor in the origin of NTD *(103,104)*. Vitamin B_{11} completely abolished the embryotoxicity of L-homocysteine in rat embryos, which was demonstrated to be mediated by catalysis of the spontaneous oxidation of L-homocysteine to the less toxic L-homocystine *(67)*. Thus, a hypothesis was developed that L-homocysteine embryotoxicity is explained by the inhibition of transmethylation reactions by increased embryonic 5-adenosylhomocysteine level.

MTHFR is a key factor in the neutralization of homocysteine, and it is a vitamin B_2 (riboflavin)-dependent enzyme. The function of thermolabile *MTHFR* is impaired because of lack of riboflavin *(105)*. The conversion of homocysteine to cystathione by cystathione synthase requires pyridoxine (a vitamin B_6-dependent enzyme). A disturbance in the above processes results in decreased homocysteine remethylation, causing hyperhomocysteinemia and the shortage of methionine; therefore, cells are not able to methylate important compounds such as proteins, lipids, and myelin. In Hungary, the daily intake of vitamin B_{12} is good, but the consumption of riboflavin and, particularly, vitamin B_6 is lower than the RDA. Additionally, vitamin C plays an important role in preventing the oxidation of THF, thus helping to keep the folate metabolic pool complete *(106)*. Folate conjugase is a zinc metalloenzyme, and folate polyglutamates are not well-absorbed by the intestine in zinc-deficient humans *(107)*. In conclusion, a diet rich in vitamins and minerals, particularly folate, is important for the prevention of NTDs and other congenital abnormalities, but cannot completely neutralize the genetic predisposition for these congenital abnormalities alone.

8.2. Periconceptional Supplementation

Good evidence is available to advise all women who are capable of becoming pregnant to have periconceptional (i.e., at least 1 mo before and until 3 mo after conception) folic acid or multivitamin (including 0.4–0.8 mg of folic acid) supplementation to reduce the occurrence of NTDs and some other major congenital abnormalities. The absorption of folic acid in the gastrointestinal tract is quick and easy. Thus, it would be

a simple and useful approach; however, about 50% of pregnancies in the United States, Hungary and many other industrialized countries are unplanned. Only the Netherlands has a 90% rate planned and/or wanted pregnancies. If women have unplanned pregnancies and have not used a supplement routinely, then they cannot take advantage of this new primary preventive method during preconceptional period.

Therefore, the use of periconceptional folic acid or multivitamins needs a strong and widespread educational campaign. However, this has resulted in only limited success. It is worth suggesting the start of the use of folic acid or multivitamins immediately after the discontinuation of oral contraceptive pills or other contraceptive methods. Unfortunately, generally, appropriate periconceptional care is not available and the practical delivery of periconceptional multivitamin/folic acid supplementation often does not occur. The establishment of periconception care is feasible and, on the basis of available experiences, economical; the network provides an appropriate opportunity for nutritional interventions as well.

Presently, there is no universal consensus of official recommendations regarding the type of supplementation and the dose of folic acid. As mentioned earlier, in 1992 the US Public Health Service *(30)* recommended that all women who are capable of becoming pregnant should consume 0.4 mg of folic acid/d to prevent NTDs. However, the following are some arguments against this recommendation.

1. The use of multivitamins containing folic acid and other B vitamins in the Hungarian RCT *(29)* and TCS *(56)* and the studies of Smithells et al. *(19–21)* showed a higher efficacy (about 90%) in the reduction of NTDs than the MRC Vitamin Study *(23)* using a high dose of folic acid alone (about 70%) and Chinese–US Study using a low dose of folic acid (41–79%) *(57)* for the prevention of NTDs.
2. Folic acid-containing multivitamins seem to be effective in the reduction of cardiovascular, urinary tract, and limb reduction congenital abnormalities; however, there are no data concerning a similar preventive effect of folic acid alone for these congenital abnormalities.
3. Hyperhomocysteinamia may play role in the origin of some NTDs; vitamin B_{12}, B_2, and B_6 are important cofactors in the folate-homocysteine metabolism, and low vitamin B_{12} is an independent risk factor in the origin of NTDs *(103,104)*.

The dose of folic acid and vitamin B_{12} also is debated. Daly et al. *(71)* did not find a dose-dependent preventive effect of folic acid for NTDs in their study, whereas Wald et al. *(108)* suggested a dose-dependent relationship between folic acid and the preventable part of NTDs in their theoretical calculation, and ecommended 5.0 mg of folic acid daily for all potentially pregnant women. This high dose may have some adverse effects in patients with pernicious anemia *(109)* and epileptic women affected with autoimmune diseases *(37)* as well. Additionally, this dose may increase the prevalence of twin births *(38–41,45)*, with a higher rate of perinatal morbidity and mortality. However, the early diagnosis of multiple pregnancies followed by specific antenatal care and delivery method can reduce the previously found risks *(45)*. In contrast, the upper tolerable dose of folate/folic acid for healthy persons, including pregnant women, is 1 mg according to the US National Academy of Sciences' Dietary Reference Intake (DRI) book *(110)*. Therefore, it is necessary to differentiate the physiological dose of folic acid (<1 mg/d) for preventive purposes and the pharmacological dose of folic acid (>1 mg/d) for the treatment of patients. The US National Academy of Sciences *(110)*

recommended that all adult men and women need 0.4 mg of folate daily, but it is crucial that women ages 19 to 50 yr ingest a further 0.4 mg of synthetic folic acid to reduce the risk of having a baby with NTDs. However, it is difficult to achieve the consumption of 0.4 mg of folate by diet, thus it is worth increasing the dose of folic acid to 0.6 mg via supplement. There is no vitamin B_{12} deficiency in Hungary and industrialized countries; however, the major concern regarding higher doses of folic acid is the so-called masking effect in patients with pernicious anemia. Therefore, the combined use of folic acid and vitamin B_{12} can prevent this possible adverse effect, but only 1 to 3% of an oral dose of vitamin B_{12} can be absorbed from the gastrointestinal tract, and a higher dose (e.g., 10 or 25 μg) of vitamin B_{12} is needed for this purpose *(111)* rather than the usual low doses (generally 4 μg).

On the other hand, folic acid is much cheaper than multiviamins. Only a few countries (e.g., Turkey) reimburse the major part of cost of multivitamins used during the periconceptional period.

8.3. Food Fortification

Food fortification seems to be the most practical means of supplementation with folic acid and other vitamins for women with unplanned pregnancies. This public health initiative is comparable to the prevention of goiter by the addition of iodine to salt.

In Ireland, vitamin B_{12} (in 1981) and folic acid (in 1987) were added to breakfast cereals, which constitute a significant part of the diet. This fortification probably contributed to the significant fall in the prevalence of NTDs at birth in the 1980s (from 4.7 to 1.3/1000), because only a small part of this decline could be attributed to termination of affected pregnancies *(103)*.

In February 1996, the US Department of Health and Human Services *(112)* ordered food fortification with folic acid of all cereal grain products at a level of 0.14 mg/100 g from January 1998. This adds only about 0.1 mg of folic acid to the average daily diet of women of reproductive age; nevertheless, the mean plasma folate concentrations increased from 4.6 to 10.0 μg/mL, and the prevalence of low folate levels (<3 μg/mL) decreased from 20 to 1.7%. Additionally, the mean total homocysteine concentration decreased from 10.1 to 9.4 μmol/L, and the prevalence of high homocysteine concentrations (>13 μmol/L) decreased from 18.7 to 9.8% *(113)*. At the same time, there was a 19 to 30% reduction in the total (birth + fetal) prevalence of NTDs *(114)*. Canada also introduced a mandatory fortification of flour with folic acid, and a 50% reduction was found in the total prevalence of NTDs *(115)*. Chile also has a mandatory flour fortification project using a higher dose of folic acid *(116)*.

In Hungary, three vitamins (folic acid [160 μg] and vitamins B_{12} [0.8 μg] and B_6 [864 μg]) are added to 100 g of flour from the third quarter of 1998 *(117)*. The daily intake of folic acid and vitamins B_{12} and B_6 is about 200, 1, and 1080 μg, respectively, in 200 g of bread. The use of vitamin B_{12} has two goals. First, it is an independent factor in the origin of NTD. Second, the oral intake of vitamin B_{12} can protect the masking effect of folic acid in patients with pernicious anemia. The consumption of fortified bread with folic acid and vitamins B_{12} and B_6 is especially important for the large proportion of women with lower levels of education and income who generally have difficulties buying food rich in folate and other vitamins and who have unplanned pregnancies. Another goal of this food fortification is the prevention of coronary artery,

cerebrovascular, and peripheral vascular diseases caused by hyperhomocysteinemia. However, plans are underway to change the too-conservative regulations concerning the doses of B vitamins in food fortification (e.g., 0.24 or 0.33 mg/100 g folic acid in flour fortification) would result in a much better benefit/cost ratio without any possible hazard if a higher dose of vitamin B_{12} (e.g., 10 or 25 μg) were introduced in flour fortification *(118)*.

9. CONCLUSION AND RECOMMENDATIONS

A considerable number of birth defects are preventable, and food fortification with folic acid and some other B vitamins appears to be the most effective method to achieve this goal. Therefore, we agree with Oakley *(119)*: inertia on folic acid fortification equals public health malpractice.

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25 Maternal Nutrition and Preterm Delivery

Theresa O. Scholl

KEY POINTS

- The rate of preterm delivery in the United States has increased by more than 25% in the past 20 yr.
- Low weight before pregnancy may be associated with increased risk for preterm delivery.
- Inadequate weight gain, particularly in the third trimester, may be associated with increased risk for preterm delivery.
- Fasting and intervals longer than 12 h between meals may be associated with increased risk for preterm delivery.
- Maternal anemia and iron deficiency anemia in the first and second trimesters may be associated with increased risk for preterm delivery.
- High hemoglobin, hemitocrit, or ferritin in the third trimester may be associated with increased risk for preterm delivery.
- Low intake of calcium, ω-3 fatty acids, antioxidants, folate, zinc, and iron may be associated with increased risk for preterm delivery.
- High maternal levels of homocysteine may be associated with increased risk for preterm delivery.
- Failure to take prenatal vitamin/mineral supplements may be associated with increased risk for preterm delivery.

1. DEFINITION AND IMPORTANCE OF PRETERM DELIVERY

The United States has an infant mortality rate that ranks 28th worldwide *(1)*. The cause of many of these early deaths is an excess of preterm deliveries to US women compared to women from countries suchs as Norway *(2)*. Preterm delivery (<37 completed weeks of gestation) is an important underlying cause of infant death *(3)*. Although congenital defects are the most important cause of mortality during the first year, in the United States, shortened gestation and low birth weight ranks as the leading cause during the neonatal period (first 28 d) and the second leading cause of death during infancy *(4)*.

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There has been a long-term decline in neonatal and infant mortality in the United States over recent decades. In contrast, the rate of preterm birth increased by 27% between 1981 (when 9.1% of all births were preterm) and 2001 (when a zenith of 11.9% was reached) *(5)*. Over the same interval, there has been less change (a 7.7% increase) in the incidence of very preterm birth (<32 completed weeks of gestation) *(5)*. The reason for the improvement in survival despite the rise in preterm delivery is better and more intensive perinatal and neonatal care, which includes procedures such as assisted ventilation and the use of corticosteroids and surfactants.

In addition to increased mortality, infants delivered preterm are at greater short-term risk of complications, including respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, and necrotizing enterocolitis, among others. In the longer term (1–3 yr), preterm children are at increased risk of seizure disorders, disabilities of the special senses (blindness and deafness), cerebral palsy, and mental retardation. Learning and behavioral deficits persist *(6,7)* and are associated with greater risks of hyperactivity, placement in special education programs, and grade retention among school children who entered life preterm *(8,9)*.

There is no way to prevent preterm delivery that is universally effective and yields consistent results. The few interventions that reduce risk are not applicable to most women or all populations. For example, treatment of bacterial vaginosis with antibiotics, even in high-risk populations, does not consistently reduce the risk of preterm delivery *(10)*. Although the injection of 17 α-hydroxyprogesterone has been shown to reduce risk, its use is limited to those women with a history of delivering before term *(11)*. Therefore, it is important to prevent preterm delivery; to do so, it is necessary to understand its etiology. This chapter examines the influence of maternal nutrition and nutritional status on risk of preterm birth.

2. RISK FACTORS ASSOCIATED WITH PRETERM DELIVERY

One problem hampering the identification of risk factors is the heterogeneous nature of preterm delivery. The most common cause is spontaneous labor, which culminates in a preterm delivery. Another etiology involves preterm premature rupture of the fetal membranes (PROM) before labor and delivery. Additionally, there are numerous complications, such as pre-eclampsia, that lead to a medically indicated preterm birth *(12)*. Each of the proximate causes may have separate risk factors. Consequently, only a fraction of the factors and exposures have been identified that give rise to preterm and very preterm delivery. These include a history of preterm delivery, poverty, African-American ethnicity, cigarette smoking, the extremes of maternal age, and inflammation and infection. Of the recognized risk factors, several implicate maternal nutrition and nutritional status (e.g., underweight, inadequate weight gain during pregnancy, anemia) as important issues regarding preterm delivery *(13)*.

3. MATERNAL WEIGHT AND WEIGHT GAIN

Most research on maternal nutritional status has focused on the relationship between pregravid weight, total weight gain, and birth weight, and the body of evidence on this topic has been reviewed extensively by the Institute of Medicine (IOM) *(14)*. Studies have been virtually unanimous (in developing countries and among different ethnic groups) in showing a positive relationship between pregravid weight or body mass

index (BMI), weight gain, and birth weight *(14,15)*. Maternal pregravid weight or BMI and weight gain appear to have independent and additive effects on birth weight. Correlations between weight gain and birth weight range between 0.20 and 0.30. The average magnitude of the effect on birth weight (in women with a normal weight-for-height) is approx 20 g of birth weight for every 1 kg of total gain, and pregravid weight-for-height is a strong effect modifier on birth weight *(16)*.

Several studies showed an association between being underweight and increased risk of preterm delivery, especially when gestation duration was estimated from fundal height or ultrasound criteria *(17–19)*. Although the finding is fairly consistent, the extent to which it represents a size bias in the estimation of gestation or to which gestational weight gain and diet during pregnancy can overcome the deficit is not well-known.

The relationship between gestational weight gain and preterm delivery appears more complex and is even more controversial. Although cumulative (or total) weight gain is an important predictor of birth weight, the pattern of weight gain and rate of gain also appear to play significant roles in predicting preterm delivery. Both early and later weight gain have independent effects on outcome during pregnancy *(20,21)*. In pregnant adolescents, an early inadequate weight gain increases the risk of small-for-gestational age infants who are below the 10th percentile for standards *(20)*. Preterm delivery appeared unaffected by early weight gain but was increased with inadequate weight gain rates (<400 g/wk) late in pregnancy. This occurred even when the total pregnancy weight gain did not fall below the targets set in clinical standards.

Other studies of adult gravidas have also indicated that low rates of weight gain, primarily in the latter half of pregnancy, are associated with preterm delivery *(17,18,22–24)*.

A study of more than 17,000 low-income African-American and Caucasian gravidas from Alabama found that rates of weight gain of less than 0.24 kg/wk after 20 wk of gestation increased the risk for preterm delivery (odds ratio [OR] = 1.52; $p < 0.05$) compared with rates between 0.58 and 0.74 kg/wk. Interestingly, Wen et al. *(17)* also noted that women with high or excessive rates of gain (≥ 0.75 kg/wk) had an increased risk of preterm delivery (OR = 1.71; $p < 0.05$), although they speculated that this association might be a function of late edema caused by pregnancy-induced hypertension (PIH). Confirming the association between low weight gain late in pregnancy and preterm delivery, Siega-Riz et al. *(18)* found, for a predominately Hispanic sample (80%) of nearly 5000 women from the West Los Angeles area, that inadequate weight gain during the third trimester was predictive of preterm delivery (more than two fold increased risk).

A third study of Alabama women *(23)* found that although low gain or low rates of gain in the first two trimesters were not associated with preterm delivery, a low rate in the third trimester (<0.38 kg/wk with a pregravid BMI < 19.8 and <0.37 kg/wk with a pregravid BMI of 19.8–26.0) was associated with an increased risk of preterm delivery (OR = 2.46, 95% confidence interval [CI] 1.53–3.92).

Most recently, Carmichael and Abrams *(24)* reviewed and summarized the extant literature (13 published studies) regarding gestational weight gain and preterm delivery. They found that 11 of these studies reported a significant association between maternal weight gain and preterm delivery—principally, risk increased when gestational weight gain was inadequate. In their summary, gestational weight gain in later pregnancy was

consistently associated with increased preterm delivery, whereas inadequate gain in early pregnancy was not.

Low rates of weight gain may not reflect maternal nutrition or may reflect it indirectly. Low intakes of iron and zinc are related to an increased risk of preterm delivery as well as to inadequate weight gain *(25,26)*. In the case of zinc, the risk of preterm delivery with low dietary zinc intake was particularly strong (threefold increased risk) for those whose rupture of membranes preceded labor *(26)*. If, for example, maternal infection increased nutrient requirements for zinc, then that infection could reduce circulating zinc levels as part of an acute phase response; infection might also increase the risk of an inadequate gestational weight gain. Conversely, maternal zinc deficiency might reduce the mother's appetite and limit weight gain while making her more susceptible to infection. Alternatively, inadequate weight gain may be a marker for maternal infection that is unrelated to maternal nutrition.

The low rates of gestational gain shown by the mother might also reflect the fact that fetuses delivered preterm grow more slowly than those delivered at term. Therefore, the mother would only seem to gain weight at a lower rate. Slowed fetal growth is found fairly consistently among gravidas who deliver preterm *(27–30)*. In Camden, New Jersey *(27)*, in a sample of young, minority mothers, we found that infants who were delivered preterm were already significantly smaller in all fetal growth dimensions by 32 wk of gestation compared with fetuses at 32 wk of gestation who were later delivered at term. The diminished fetal growth occurred from about 16–32 wk. When stratified by the proximate cause of preterm delivery, infants delivered preterm for medical or obstetric indications (placental abruption, severe pre-eclampsia, nonreassuring heart rate patterns, chorioamionitis, oligohydramnios) had asymmetrical growth patterns, which suggested a growth failure late in pregnancy. On the other hand, infants delivered preterm after PROM or after failed or no tocolytic therapy to halt their spontaneous preterm labor were proportionately smaller in all dimensions, implying an overall slowing of growth that may have originated early in pregnancy that may have been caused by a more chronic stress.

4. DIET AND GESTATIONAL WEIGHT GAIN

Maternal diet is one reason that maternal weight gain during pregnancy and gestation duration may be positively correlated. Although this association appears to be reasonable, a relationship between energy intake and weight gain during pregnancy has not been described often.

The first report of a positive relationship between diet and weight gain was made by Thomson *(31)*, who found a correlation of 0.30 between intake and weight gain in Scottish primigravidas eating "to appetite." Among Camden gravidas, a significantly lower energy intake (about 300 kcal/d less) was associated with an inadequate gestational gain *(32)*. After controlling for energy, women with an inadequate gestational weight gain showed little difference in macronutrients (protein, calories) or total grams of food ingested. This relationship was subsequently confirmed with three 24-h dietary recalls taken during the course of pregnancy *(26)*. Because intakes were obtained throughout gestation, overall intake was higher (+160 kcal/d) than in the prior study. After controlling for confounding variables, women with inadequate gestational weight gain consumed fewer kcal per day (–173 kcal/d) than women whose pregnancy weight gain was adequate. Recently, women

from upstate New York were asked a series of behavioral questions regarding changes in food intake during pregnancy; results from these questions confirmed and extended these findings *(33)*. Consuming "a little or a lot less food" during pregnancy was associated with a greater than twofold increase in weight gain below IOM guidelines. Conversely, eating "much more food" was associated with a twofold increased risk of an excessive weight gain. By comparison, a reduction in physical activity was associated with a 70% increase in the risk of an excessive weight gain.

5. DIET AND PRETERM DELIVERY

The relationship between poor diet and inadequate gestational weight gain and the observation that slowed fetal growth, low prepregnant weight or low BMI, and low rates of weight gain were each associated with preterm delivery suggests that a poor maternal diet may be a risk factor. During the Dutch Famine of 1944–1945, exposure to intense famine in the third trimester shortened gestation by about 4 d, whereas exposure during the first trimester was associated with a clear excess of preterm birth *(34)*. The famine was better known for its effect on fetal growth and maternal weight. The identification of preterm births probably was made difficult by famine-related amenorrhea, which would have made gestational dating less secure for many women.

Fasting during pregnancy may produce effects analogous to famine. In sheep, a short interval of food deprivation around the time of conception–(from 2 mo before to the 1 mo after conception) increased the risk of preterm birth. The nutritional restriction was brief: it reduced maternal weight by about 15% and was followed by *ad libitum* feeding for the remainder of gestation *(35)*. Because the nutritional demands of the fetus were modest early in gestation, the trigger of preterm delivery was unclear. The authors speculated that the shortage of an essential nutrient(s) was a more likely cause than a lack of calories *(36)*.

Later in pregnancy, a fast of 24 to 48 h elicits labor and/or delivery, and this has been demonstrated across species in ewes *(37,38)*, mares *(37–39)*, and primates *(40)*. In these animals, maternal starvation reduced circulating glucose levels and increased free fatty acid production; these, in turn, correlated with higher fetal cortisol and greater prostaglandin production by both mother and fetus *(40)*. Fasting may elicit a similar response in humans: abstaining from food and water during the Yom Kippur fast is associated with an increased risk of labor onset for women who are close to term *(41)*. In addition to lower prepregnancy weight and weight gain, one study suggested that women in preterm labor were more likely to have detectable ketone bodies than controls *(42)*. Although this could mean that women eat less early in labor, it is also possible that many of the pregnant women may have been experiencing so-called "accelerated starvation" and may have been metabolizing fat stores *(43)*.

A pattern of infrequent eating and snacking throughout the day also may increase risk for preterm birth. In one study, women who consumed less than three meals and two snacks during the third trimester increased their risk of preterm delivery by 30% *(44)*. Similarly, periods of extended fasting–(13 h or more during the second and third trimesters) were associated with increased levels of corticotropin-releasing hormone (CRH) *(45)*. In this and other studies, higher CRH concentrations correlated with shorter gestation duration and an increased risk of preterm delivery *(46)*. Therefore,

famine and fasting—perhaps in concert with low levels of maternal glucose and other circulating nutrients—may influence CRH production and increase the risk of preterm delivery.

6. IRON

Iron is as essential element in the production of hemoglobin for the transport of oxygen to tissues and in the synthesis of enzymes that are required to use oxygen for the production of cellular energy *(14)*. Supplementation with iron generally is recommended during pregnancy to meet the energy needs of both the mother and the rapidly growing fetus. Anemia (low hemoglobin levels) and iron deficiency anemia (IDA) sometimes serve as indicators of overall poor maternal nutritional status during pregnancy. Anemia appears to be one of the most obvious symptoms when overall dietary intake is inadequate. However, not all anemia is nutritional in origin—some arises from infection.

When detected early in pregnancy, IDA is associated with a lower energy and iron intake, an inadequate gestational weight gain over the whole of pregnancy, and a greater than twofold increase in the risk of preterm delivery *(25,47)*. Maternal anemia, when diagnosed before midpregnancy, is also associated with an increased risk of preterm birth *(48–50)*. During the third trimester, anemia may be a good prognostic sign reflecting expansion of the maternal plasma volume. Therefore, during the third trimester, anemia often is associated with decreased, rather than increased, risk of preterm birth *(51)*.

Scanlon and colleagues *(52)* used data from Pregnancy Nutritional Surveillance to examine the relationship between maternal anemia and preterm delivery in 173,031 low-income gravidas. Preterm delivery was increased for anemic women and women with low hemoglobin during the first or second trimester. For women with moderate-to-severe anemia, risk was approximately doubled; for the others, risk of preterm delivery was increased between 10 and 40%. During the third trimester, the association reversed: anemia and low hemoglobin each were associated with a decreased risk of preterm birth, and there was little relationship between high maternal hemoglobin and preterm delivery *(53)*. The association of maternal anemia in early pregnancy with preterm delivery and other poor outcomes was confirmed in recent studies from the developing world, including China, Nepal, and Egypt *(51,54,55)*.

The increased risk of preterm delivery may be specific to IDA and not anemia from causes other than iron deficiency. Scholl *(25)* reported data from 755 pregnant women receiving initial antenatal care at 16.7 ± 5.4 wk of gestation in Camden. Serum ferritin <12.0 μg/L) was used to characterize iron deficiency anemia *(14)*. Although anemia based on low hemoglobin was high (27.9%) at the initial antenatal visit, prevalence IDA (anemia with serum ferritin concentrations <12.0 μg/L) was low, amounting to 3.5%. After controlling for confounding variables, for women with IDA early in gestation, the risk for preterm delivery increased more than twofold, whereas anemia from other causes was not associated with any increased risk for preterm delivery. Consistent data were obtained from Papua, New Guinea. Severe anemia (<80 g/L) in early pregnancy (which was attributed to iron deficiency) increased risk of low birth weight about sixfold among primiparous women. Risk was not increased when IDA was diagnosed at delivery *(55)*.

Thus, it seems reasonable to presume that some, but not all, of what appears to be anemia or IDA is caused by the expansion of the maternal plasma volume. Currently,

this state is poorly differentiated from anemia or IDA late in pregnancy but is easier to distinguish during the first or second trimester.

Later in pregnancy, high levels of hemoglobin or hematocrit are associated with an increased risk of preterm delivery. High hemoglobin levels often correlate clinically with increased risk of pre-eclampsia and suggest failure of the plasma volume to expand. Numerous studies have documented such a "U-shaped" relationship between low and high maternal hemoglobin or hematocrit and preterm delivery (Table 1). Garn et al. *(56)* noted the relationship in data from the National Collaborative Perinatal Project. Using the lowest recorded pregnancy values of hemoglobin and hematocrit, they demonstrated an increased risk of preterm delivery with hemoglobin levels less than 100 g/L and greater than 120 g/L. Murphy et al. *(57)* studied nearly 55,000 women from the Cardiff Births Survey and found the same "U-shaped" relationship at entry to prenatal care (booking). Risk of preterm birth was increased with low hemoglobin levels (<104 g/L) for women entering care before 13 wk and after 20 wk of gestation, whereas for late entrants to care (weeks 20–24), risk was also increased when hemoglobin levels were high. In a multiethnic study of moer than 150,000 women from the North West Thames region of London, Steer et al. *(51)* demonstrated an increased risk of preterm delivery with both low (≤85 g/L) and high (<115 g/L) hemoglobin levels among all ethnic groups. Although African, Afro-Caribbean, and Indo-Pakistani women had higher rates of preterm delivery, their higher rates of anemia did not account for the increased risk.

A high concentration of the iron storage protein ferritin during the third trimester of pregnancy also is associated with an increased risk for preterm and very preterm delivery. In studies of Alabama women, Goldenberg *(58)* found that high levels of serum ferritin (>40 ng/L) during the third trimester was a marker for an increased risk for preterm and very preterm delivery. There is a trend for serum ferritin to rise with advancing gestation in women who deliver preterm *(59)*. In Camden *(60)*, high ferritin levels (>41.5 ng/L) during the third trimester (stemming from the failure of ferritin to decline as plasma volume expands) increased risk of very preterm delivery more than eightfold. High ferritin has been associated with indicators of maternal infection, including clinical chorioamnionitis *(60)* and infant sepsis *(61)*. Maternal anemia and IDA earlier in pregnancy with inadequate expansion of maternal plasma volume appeared to underlie high ferritin during the third trimester in the Camden study *(60)*. In studies of nonanemic gravidas, high levels of maternal ferritin correlated with maternal iron status but not infection *(62)*. A recent clinical trial of supplemental iron among nonanemic women participating in Women, Infants and Children program produced a puzzling result: there were increases in birth weight and gestation duration in the absence of change in maternal iron status *(63)*. Prophylactic iron supplementation from entry (before week 20) to week 28 did not increase serum ferritin or reduce risk of either maternal anemia or IDA or any other measures of maternal iron status in iron-supplemented women compared to controls. However, women who received iron (as 30 mg/d of ferrous sulfate) had significantly longer gestation duration (0.6 wk), increased infant birth weight (206 g), and decreased risks of infant low birth weight and preterm low birth weight. Risk of preterm delivery (reckoned from the mother's unconfirmed last menstrual period alone) was not reduced by supplementation with iron.

The reason that maternal anemia in the first and second trimesters is associated with an increased risk of preterm delivery is not known. Allen *(64)* suggested that the underlying mechanism(s) possibly involve alterations in the growth and development of the

Table 1
Maternal Nutrition and Preterm Delivery, Study Characteristics

Nutrient	*Reference*	*Study design, size, and setting*	*Age (yr)*	*Socioeconomic status*	*Race/Ethnicity*	*Findings*
Iron/Anemia	Garn et al. *(56)*	Observational study of over 50,000 women followed in the National Collaborative Perinatal Project (NCPP), lowest pregnancy values of hemoglobin (Hgb) and hematocrit (Hct).	—	Mixed	Caucasians and African-Americans	Maternal Hgb and Hct levels had "U-shaped" relationship to preterm delivery (≤37 wk), with the risk being higher at Hgb <100 g/L and >120 g/L. The increased risk with Hgb indicated a failure of plasma-volume expansion.
	Murphy et al. *(57)*	Observational study of nearly 55,000 women in the Cardiff Births Survey, Hgb levels ascertained at entry to prenatal care (booking).	—	Mixed	Welsh	Preterm delivery (<37 completed weeks) showed a U-shaped" relationship to Hgb levels. At <13 wk, risk was increased about 50% with Hgb < 104 g/L; at weeks 13 to 19 risk was not increased; at weeks 20 to 24, risk was increased for low (104 g/L) and high (>145 g/L) Hgb by over 50%.

Klebanoff et al. *(49)*	Case–control study of 1706 (725 preterm, 981 term) deliveries from Kaiser Permanente Births Defects Study, Hct levels measured throughout gestation.	—	—	14% Asian 28% African-American 21% Mexican 37% Caucasian	Hct values fell during early second trimester and began increase again at 31 to 33 wk. Moderate relationship between second trimester anemia (Hct < 10th percentile for ethnicity and gestation) and preterm delivery.
Steer et al. *(51)*	Retrospective analysis of more than 150,000 women from the North West Thames region of London, lowest recorded Hgb during pregnancy.	—	Mixed	73% Caucasian 14% Indo Pakistani 5% African-American 8% Other	Increased risk of preterm delivery (<37 wk completed) with Hgb levels ≤85 g/L (AOR = 1.62, CI95%:1.35–2.18) and >115 g/L.
Zhou et al. *(50)*	Observational study of 829 women from Shanghai. Population homogeneous for race, parity, prenatal care, smoking. Hgb sampled at 4–8, 16–20, and 28–32 wk of gestation.	27.7 ± 4.7 yr	—	Chinese	Inverse "U-shaped relationship" between maternal Hgb at 4–8 wk and preterm delivery and low birth weight. Odds of preterm delivery increased 2.5-fold for women with Hgb > 130 g/L or between 90 and 99 g/L. Odds increased 3.5-fold for entry Hgb between 60 and 89 g/L. Risk of fetal growth retardation unrelated to entry Hgb

(*Continued*)

Table 1 (*Continued*)

Nutrient	*Reference*	*Study design, size, and setting*	*Age (yr)*	*Socioeconomic status*	*Race/Ethnicity*	*Findings*
	Scanlon et al. *(52)*	Retrospective analysis of data from 173,631 pregnant WIC participants.	70% 20–35 yr	Low	55% Caucasian 20% African-American 19% Hispanic 6% Other	Risk of preterm delivery increased with low Hgb in first and second trimester. Severe anemia 1–68 SGA not associated with anemia. AOR of preterm delivery with first trimester severe anemia increased 1.7 times.
	Cogswell et al. *(63)*	Randomized, placebo-controlled trial of WIC participants, not anemic at entry (<20 wk). Participants supplemented until wk 28, 30 mg FE as ferrous sulphate or placebo. At wk 28 controls with low ferritin received iron (30 or 60 mg/d).	24.3 ± 5 yr	Low	56% Caucasian 25% African-American 16% Hispanic 3% Other	Iron supplementation did not reduce anemia or increase ferritin or other iron status measures. Iron supplementation increased birth weight (206 g) and decreased risk of low birth weight and preterm low birth weight but not preterm delivery.
IDA	Scholl et al. *(25,47)*	Observational study of IDA (IDA, anemia with serum ferritin <12 μg/L) among 779 women from Camden, NJ.	18.4 ± 3.7 (anemia) 18.4 ± 3.9 (no anemia)	78% Medicaid (anemia)	77% African-American (anemia) 56% African-American (no anemia)	IDA (found in 12.5% of those with anemia) was found to be associated with lower energy and iron intakes early in pregnancy. The risk or preterm delivery was

							better than two times with IDA, but was not increased with anemia from other causes.
	Iron/Ferritin	Goldenberg et al. *(58)*	Observational study of serum ferritin at 19, 26, and 36 wk and preterm delivery in 580 gravidas who participated in a randomized study of zin supplementation	—	100% Low	100% African-American	Ferritin in the highest quartile at week 28 was associated with a twofold increase in odds or preterm (<37 wk) delivery and infant low birth weight, a threefold increase in odds of very preterm (≤32 wk) delivery, and a fourfold increase in birth weight ≤1500 g.
		Scholl *(60)*	Observational study of serum ferritin (15 and 28 wk) in 1162 gravidas from the Camden Study.	12 to 29 yr	100% Low	33.3% Hispanic 57.3% African-American 9.4% Caucasian	At week 28 high concentrations of ferritin (>90th from failure of gestation was associated with a ninefold increase in very preterm delivery, a fourfold increase in preterm delivery, and a fivefold increase in low birth weight ($p < 0.01$ for each).
		Goldenberg et al. *(61)*	Observational study of 223 gravidas with PROM before 32 wk. Data from randomized	24.8 ± 5.9 yr	—	American	Plasma ferritin increased between admission for PROM and delivery. Ferritin levels were

(*Continued*)

Table 1 (*Continued*)

Nutrient	*Reference*	*Study design, size, and setting*	*Age (yr)*	*Socioeconomic status*	*Race/Ethnicity*	*Findings*
		antibiotic trials at five clinical centers.				significantly higher (by 36 μg/L) in women whose infants developed sepsis.
Zinc	Cherry et al. *(71)*	Randomized, controlled trial of zinc supplementation among pegnant teenagers from New Orleans who were assigned to placebo ($n = 556$) or 30 mg of zin supplementation ($n = 581$)	17.6 yr (range: 13.5 to 19.6 yr)	Low	95% African-American	With stratification by maternal weight at delivery, zinc supplementation was related to a lower risk of preterm delivery (38 wk) among normal-weight women (18.3 vs 28%; $p < 0.05$). Zinc-supplemented low-weight multiparas had increased gestation duration (+1.2 wk; $p < 0.008$). There was a tendency ($p < 0.15$) for weight gain rates to be greater among zinc supplemented women (0.52 kg/wk) compared with placebo (0.42 kg/wk).
	Goldenberg et al. *(72)*	Randomized, controlled trial of zinc supplementation among pregnant Alabama	23.9 ± 5.5 yr (zinc) 22.8 ± 5.4 (zinc)	Low	100% African-Americans	Birth weight increased with zinc supplementation by 126 g ($p < 0.05$) and

	women with plasma zinc below median at entry to prenatal care, assigned to multivitamin tablet with an additional 25 mg of zinc ($n = 294$) or placebo ($n = 286$)				gestation duration by 0.5 wk ($p = 0.06$). Women with pregravid BMI less than 26 had a birth weight increase of 248 g ($p < 0.005$) and an increase in gestation of 0.7 wk ($p = 0.08$).
Neggers et al. *(68)*	Observational study of 476 Alabama women.	21.8 ± 4.9	Low	76.5% African-American	Low plasma zinc at entry to prenatal care (7.0–12.2 μmol/L) increased risk of infant low birth weight eight times compared with women in the highest quartile (15.9–25.4 μmol/L; $p < 0.05$). Plasma zinc was linearly related to gestation duration (0.17 wk/μmol/L zinc; $p < 0.05$).
Scholl et al. *(26)*	Observational study of dietary zinc intake in 818 pregnant women from Camden, NJ.	19.2 (≤ 6 mg of zinc per day) 18.7 (>15 mg of zinc per day)	Low	60% African-American	Low dietary zinc intake associated with twice greater preterm (<37 wk) delivery and infant low birth weight, three to four times greater very early preterm (<33 wk) delivery ($p < 0.05$ for each). Risk for preterm delivery with

(*Continued*)

Table 1 (*Continued*)

Nutrient	*Reference*	*Study design, size, and setting*	*Age (yr)*	*Socioeconomic status*	*Race/Ethnicity*	*Findings*
						PROM increased 3.5 times with low zinc ($p < 0.05$).
	Sikorski et al. *(70)*	Observational study of zinc status in 70 women with term deliveries.	26 ± 4.4 yr	—	Polish	Zinc index compiled from zinc assayed in blood, scalp and pubic hair and colostrum. Zinc index in patients with PROM was lower (4.33 ± 1.18) compared with patients without PROM (5.97 ± 1.39; $p < 0.05$). Zinc index was inversely correlated with parity ($r = –0.61$).
Folic acid/ folate	Tchernia et al. *(87)*	Observational study of serum folate in 100 women using iron or iron and vitamin C.	—	—	French	Serum folate lower by 1.1 µg/L in women delivering ≤39 wk ($p < 0.01$).
		Observational study of red cell folate in 100 high-risk women.	—	—	French	Gestation duration reduced by 0.8 wk in women with RBC folate ≤200 µg/L ($p < 0.025$).
		Supplementation trial (open) of iron vs iron + folate (350 µg/d) in 108 women.	—	—	French	Gestation duration increased by 0.8 wk among iron and folate supplemented women ($p < 0.001$).

	Blot et al. *(86)*	Nonrandomized, double-blind study of iron and ascorbic acid vs iron, ascorbic acid, and folate at 6 mo of gestation in 200 women.	27.5 ± 4.5	34% Low	French	Gestation duration increased by 0.8 wk with folate (350 µg/d) supplementation, birth weight increased by 158 g, birth length by 1.7 cm ($p < .05$).
	Scholl et al. *(65)*	Observational study of folate from diet and supplements and serum folate in Camden, NJ ($n = 832$).	18.8	Medicaid 80%	67% African-American	Women with low folate intake (≤240 µg/d) greater preterm delivery and had three times infant LBW ($p < 0.05$). Risk of preterm delivery without PROM increased three times ($p < 0.05$). Odds of preterm delivery increased 1.5% per unit decrease in serum folate ($p < 0.05$).
Folic acid	Giles et al. *(85)*	Randomized study of folic acid (5 mg/d) or iron supplementation in 692 gravidas. Patients stratified by gestation at entry to care: <10 wk, 10–20 w, 20–30 wk, > 30 wk.	24.9 ± 5.2 yr (iron) 25.1 ± 5.7 yrs. (folic acid + iron)	—	Australian (Melbourne)	Folic acid group contained more primigravidas than placebo group. No difference between groups in gestation duration or birth weight.
	Fletcher et al. *(84)*	Double-blind, randomized study of folic acid plus	27.2 ± 5.7 yr (folic	—	English	Gestation duration (39.7 wk [London])

(*Continued*)

Table 1 (*Continued*)

Nutrient	*Reference*	*Study design, size, and setting*	*Age (yr)*	*Socioeconomic status*	*Race/Ethnicity*	*Findings*
		iron (5 mg/d) or iron in 643 gravidas.	acid + iron) 27.4 ± 5.7 yrs. (iron)			iron vs 39.7 folic acid + iron) and proportion less than 38 wk (8.7 vs 8.7%) were not different.
	Fleming et al. *(83)*	Randomized controlled trial of folic acid (0.5 mg or 5 mg/d) with and without iron plus a placebo group in 146 gravidas.	—	—	Western Austrialia	Small sample sizes in each of the five trial arms ($n \sim 20$). No difference in gestation or birth weight was detected among groups.
Folic acid/ homocysteine	Malinow *(92)*	Observational study of circulating serum homocysteine and serum folate in 35 healthy nulliparous gravidas at delivery.	24.2 ± 5.9 yr	—	American	In univariable analyses, maternal homocysteine correlated significantly ($p < 0.05$) withgestation duration ($r = –0.42$) serum folate correlated significantly ($p < 0.05$) with gestation ($r = 0.23$).Maternal folate and homocysteine correlated significantly ($r = 0.54$) with each other ($p < 0.05$). Maternal serum B_{12} was not related to gestation duration ($r = 0.08$; $p = 0.67$).

	Rajkovic *(89)*	Observational study of plasma homocysteine in 20 nulliparous gravidas with pre-eclampsia and 20 controls.	20 ± 4 yr	—	45% African-American 55% Caucasian	Pre-eclamptic gravidas had higher homocysteine (8.66 vs 4.99 g mol/L), and delivered significantly earlier than controls (35 ± 4 vs 40 ± 1 wk) ($p < 0.05$ for each).
	Vollset et al. *(90)*	Retrospective cohort analysis of 5883 women linking current homocysteine to past pregnancy outcomes.	40–42 yr	—	Norwegian	Current homocysteine (highest quartile) associated with history of adverse pregnancy outcome including pre-eclampsia, preterm delivery, low birth weight, stillbirth, neural tube defects and club foot.
	Ronnenberg et al. *(91)*	Case–control study of prepregnancy homocysteine B vitamins (B_6, B_{12}, folate) and pregnancy outcome in 405 women from Anquin, China.	24–27 yr (23.0–34.2)	—	100% Chinese	Risk of preterm delivery increased 3.6-fold for women with elevated homocysteine (≥12.4 μμ/L). Preterm delivery, 50–60% lower for women with nondeficient levels of B_{12} and B_6. Serum folate not associated with preterm delivery.
Calcium	Lopez-Jaramillo et al. *(99,100)*	Randomized, controlled trial of calcium supplementation among 56 nulliparas at risk	19.4 ± 1.8	Low-income Ecuadorian clinic patients with overall		Gestation duration longer for 22 supplemented women (2 g calcium/d) vs placebo group

(*Continued*)

Table 1 (*Continued*)

Nutrient	*Reference*	*Study design, size, and setting*	*Age (yr)*	*Socioeconomic status*	*Race/Ethnicity*	*Findings*
		for pregnancy-induced hypertension in Quito, Ecuador.		low calcium intake.		(n = 34). Gestation 39.2 ± 1.2 wk for calcium supplemented, 37.4 ± 2.3 wk for placebo ($p < .01$).
	Villar and Repke *(101)*	Randomized, controlled trial of calcium supplementation, 190 teenagers in Baltimore, MD.	≤17	Low-income clinic patients, 93.7% African-American		Gestation duration longer for 94 supplemented teenagers (2 g calcium per day) vs placebo group (n = 95). Gestation 39.2 ± 2.7 wk for calcium supplements, 37.9 ± 3.9 wk for placebo ($p < .01$) Lower percentage of preterm delivery (<37 wk) among supplemented (7.4%) vs placebo (21.1%; $p < 0.01$).
	Belizan et al. *(96)*	Randomized, controlled trial of calcium supplementation among1194 gravidas in Rosario, Argentina.	23.7 ± 5.5 (calcium) 23.7 ± 5.7 (placebo)	Mixed	Argentinian	Percent with preterm delivery(<37 wk) among 579 calcium-supplemented women (2 g calcium/d) 6.3 vs 6.8% for 588 women receiving placebo.
	Bucher et al. *(102)*	Medline and Embase searches for	—	Mixed	—	The summary odds ratio for preterm delivery

	randomized trials of calcium and pre-eclampsia during pregnancy (2459 subjects enrolled in 14 trials) for meta-analysis.				was 0.69 (95% CI 0.48–1.01) and not statistically significant. While all the trials which included preterm delivery showed a reduced risk with calcium supplementation during pregnancy only one was statistically significant.
Levine et al. *(103)*	Randomized trial of calcium supplementation (500 mg/d) or placebo in 4589 healthy nulliparas at13–21 wk gestation. Eligible women underwent test of compliance before enrollment in trial.	21 ± 4 yr	Mixed	35% Caucasian 17% Hispanic 47% African-American 2% Other	Calcium-supplemented gravidas delivered at 38.9 ± 2.5 vs 38.9 ± 2.4 wks for placebo (NS). Preterm delivery (<37 wks) and very preterm delivery (<34 wk) occurred in 10.8 and 4.2% of supplemented vs 10.0 and 3.7% of controls (NS). low birth weight (<2500 gm) was 8.8% in supplemented, 9.6% in controls (NS).
Crowther et al. *(104)*	Randomized trial of calcium supplementation (1.8 g/d) or placebo in 456 nulliparous with singleton pregnancies.	25 ± 5 yr	Mixed	Australian	Treatment with calcium reduced risk of preeclampsia and preterm birth with more than two fold reduction in risk for each.

(*Continued*)

Table 1 (*Continued*)

Nutrient	*Reference*	*Study design, size, and setting*	*Age (yr)*	*Socioeconomic status*	*Race/Ethnicity*	*Findings*
Fish oils (*n*-3 fatty acids)	Olsen et al. *(105)*	Randomized, controlled trial of supplementation with fish oil, 533 women assigned 2, 1, and 1 to fish oil, olive oil, or no oil supplementation, respectively, in Aarhus, Denmark.	29.4 ± 4.4 (fish oil) 29.7 ± 4.3 (olive oil) 29.1 ± 4.1 (no oil)	—	Danish	Mean gestation duration longer ($p < 0.006$) for the 266 women receiving fish oil (283.3 ± 11.1 d) compared with olive oil (279.4 ± 13.1 d) or no oil groups (281.7 ± 11.6 d).
Multivitamin/ Minerals	Scholl et al. *(112)*	Observational study of 1430 gravidas from the Camden Study who entered care during trimesters one and two. Prenatal supplement users compared to gravidas entering care in same time frame who did not use supplements.	12–29 yr	Low	58.9% African-American	Prenatal supplement use associated with two- to fourfold reduction in odds of preterm delivery and very preterm delivery ($p<.001$). Significant reductions in odds of very LBW (six- to sevenfold) and infant low birth weight (twofold) ($p < 0.001$). Prenatal supplement use corroborated by assay of circulating micronutrients at 15 and 28 wk ($p < 0.05$).

Fawzi et al. *(115)*	Randomized study of 1075 HIV-1-infected gravidas from Tanzania randomly assigned to placebo, vitamin A, or multivitamins in a 2×2 factorial design	—	Low	Tanzanian	Women receiving multivitamins had significantly decreased ($p < 0.01$) infant low birth weight by (44%), very preterm birth (39%), and fetal growth restriction (43%) CD4, CD8, and CD3 counts were significantly increased ($p<0.05$). Vitamin A had no effect on these outcomes.
Wu et al. *(114)*	Livebirths from 1988 NMIH Survey (n ~ 9000) analyzed to examine effect of regular use of multivitamin/mineral supplements and smoking. Gravidas regularly using supplements (3+ times/wk) compared to those who used them less often.	25.5 ± 5.9 yr	Mixed	American	No difference between smokers who regularly used multi-vitamins and those using them less in odds of preterm delivery, LBW or fetal growth retardation.
Christian et al. *(116)*	Cluster randomized controlled trial of 426 rural communities in SE Nepal. Communities randomized to five	55% 20–29 yr	Low	Nepalese	None of the supplements reduced risk of preterm delivery. In comparison to vitamin A control, multiple micronutrients increased birth weight

(*Continued*)

Table 1 (*Continued*)

Nutrient	*Reference*	*Study design, size, and setting*	*Age (yr)*	*Socioeconomic status*	*Race/Ethnicity*	*Findings*
		regimes of supplementation vs vitamin A control.				(+64 g) and reduced low birth weight by 14%. Folic acid plus iron also increased birth weight (+37 g, NS) and reduced low birth weight by 16%.
	Ramakrishnan et al. *(117)*	Randomized trial of multivitamin supplements vs iron.	23 ± 5 yr	Low	Mexican	Multivitamin and iron supplements did not yield differences in birthweight, gestation duration, low birth weight, IUGR, or ponderal index.
Antioxidants	Chappell et al. *(120,121)*	Randomized placebo controlled trial of supplementation with antioxidant vitamins C and E in gravidas (n = 283) at risk of preeclampsia.	29 ± 5	Mixed	61% European 21% African 15% Caribbean 3% Other	Supplements with vitamins C and E reduced risk of preeclampsia 2.5-fold. No differences in preterm delivery, SGA reduced by 28% (NS).
	Sharma et al. *(122)*	Randomized placebo controlled trial of supplementation with lycopene in New Delhi primparas (n = 251).	22	Mixed	Indian	Supplementation with lycopene reduced risk of preeclampsia by 50%, reduced diatolic blood pressure, and risk of SGA (by 50%) and increased gestation duration by 1 to 2 wk.

placenta and fetus that are related to hypoxia. Chronic hypoxia activates the stress response and, in theory, the release of CRH by the placenta, the increased production of cortisol by the fetus, and an early delivery. Another plausible mechanism involved increased oxidative stress in iron-deficient women that was not offset by endogenous or dietary antioxidants, with subsequent damage to the maternal–fetal unit *(64)*. A third credible hypothesis involved infection, that is, reduced immune function and increased risk of infection among iron-deficient women, with increased production of inflammatory cytokines, secretion of CRH, and production of prostaglandins *(64)*.

7. MICRONUTRIENTS

During pregnancy, low intakes of two micronutrients—zinc *(26)* and folate *(65)*—are associated with lower energy intake, IDA at entry to care, an inadequate gestational gain during pregnancy, and an increased risk of preterm delivery. Zinc is an element involved either directly (as a metalloenzyme) in the production of enzymes, including DNA and RNA polymerase, or as a catalyst in the synthesis of other enzymes *(66)*. Folic acid functions as a coenzyme in the transfer of single-carbon atoms to intermediates in the synthesis of amino acids and nucleic acids *(67)*. Although many other nutrients also would be limited in a marginal maternal diet, inadequate intake of zinc, folate, or both potentially leads to impaired cell division and alterations in protein synthesis. Such alterations are most notable and have the greatest potential to do harm during times of rapid tissue growth, such as pregnancy *(14)*.

7.1. Zinc

Neggers et al. *(68)* used plasma zinc as an indicator of zinc status and demonstrated a positive association between the maternal serum zinc at 16 wk of gestation and birth weight in a large population of low-income women from Alabama. Serum zinc in the lowest quartile was associated with an eightfold increased risk of low birth weight, suggesting a threshold effect for circulating levels of zinc during pregnancy (Table 1). Kirksey et al. *(69)* examined the relationship of maternal zinc nutriture to birth weight and found that plasma zinc concentrations in the second trimester, along with pregnancy weight at 3 mo of gestation, formed the best predictor model of birth weight for these pregnancies, accounting for 39% of the variance. Scholl and colleagues *(26)* examined the relationship of dietary zinc intake in 818 Camden women and reported increased risks of complications and adverse pregnancy outcomes in association with low intakes of dietary zinc (6 mg/d or less). These complications included increased inadequate gestational weight gain and a greater risk of IDA at entry to care. Poor outcomes associated with low zinc intake included risks of low birth weight, preterm delivery, and very preterm delivery two-to-three times greater than controls. In the presence of IDA, the risk of very preterm delivery was greater than fivefold. Sikorski et al. *(70)* also reported a lower zinc index at delivery (composite of zinc in hair, colostrum, and plasma) in women with premature rupture of the membranes at term (Table 1).

Although observational studies have been suggestive, clinical trials of zinc supplementation have focused on entire groups of low-income women in which the mean zinc intake is below the Recommended Daily Allowance (RDA) for pregnancy. These trials have yielded equivocal results, perhaps because of an approach that selects a population, rather than individuals, at risk.

Two trials showed effects of zinc that were conditional on maternal weight *(71,72)*—that is, a lower rate of preterm delivery in zinc-supplemented women who were not overweight (Table 1). Cherry et al. *(71)* reported a response to zinc in pregnant adolescents supplemented from before 25 wk of gestation. Low serum zinc concentrations were more common in underweight and multiparous women. The frequency of preterm delivery was reduced in the zinc-treated normal-weight women compared to the placebo group. Zinc treatment of underweight multiparous women also was associated with a gestational age increase of nearly 3 wk.

The trial conducted by Goldenberg and colleagues *(72)* took a more targeted approach and recruited women with plasma zinc levels below the median and randomly assigned them to zinc or placebo. The analysis was stratified by BMI (BMI ≥ 26 and BMI < 26). Zinc supplementation was associated with increased gestation duration of approximately half a week ($p = 0.06$) and an increase in birth weight (about half of which was explained by the longer duration of gestation). Women with a BMI <26 benefited most from zinc treatment with a 248-g increase in infant birth weight and a 0.7 cm-larger infant head circumference. Consistent with earlier results, effects were increased for women with lower pregravid BMI (Table 1).

In contrast, two recent trials from the developing world (Peru, Bangladesh), where one might suppose that zinc deficiency would be prevalent, were negative *(73,74)*. It is possible that if gravidas from the developing world have multiple nutritional deficiencies, then these may reduce the bioavailability of zinc or otherwise limit fetal growth.

However, low plasma zinc has a number of potential causes. In addition to its effect on protein synthesis, zinc also has an antiseptic action. In theory, a low zinc intake could be associated with an increased risk of infection during pregnancy, leading to fragile fetal membranes (and PROM). A genetic polymorphism in matrix metalloproteinase-9, one of the genes that codes for zinc-dependent enzymes, was recently associated with an increased risk of PROM *(75)*. Conversely, a low plasma zinc level could be an acute phase response to a stressor, such as maternal infection *(66)*. Keen *(76)* recently commented on the importance and need for distinguishing a secondary zinc deficiency arising as an acute phase response to infection or inflammation from a primary (dietary) deficiency in zinc.

7.2. Folate

During gestation, marginal maternal folate nutriture has the potential to impair cellular growth and replication in the fetus and/or the placenta, which in turn could increase the risk of preterm delivery and infant low birth weight *(14)*. Such adverse outcomes are more prevalent among poor women than those who are affluent. Pregnant women living under circumstances in which preterm delivery is prevalent have been reported to consume diets with a lower density of vitamins and minerals, including folate *(77)*, and to limit consumption of folate-containing dietary supplements *(78,79)*.

In the United States, it is recommended that women consume 600 µg of folic acid per day during pregnancy (which includes 400 µg of synthetic folic acid from supplements or fortified cereals) to reduce risk of neural tube defects (NTDs). Unfortunately, less than one-third of women of childbearing age follow this recommendation *(80)*. Also in the United States, fortification of flour and cereals with folic acid (1998–present) has been associated with a 19% decline in the risk of liveborn infants with NTDs along with increases in serum and red cell folate and a decline in homocysteine levels *(81,82)* (*see* Chapter 24).

The influence of dietary and circulating folate on preterm delivery and infant low birth weight was studied in 832 women from Camden, one of the poorest cities in the continental United States. *(65)*. Low intakes of folate from diet and supplements were associated with maternal characteristics reflecting poorer maternal nutritional status, including lower energy intake, low rate of gestational weight gain, and a higher frequency of IDA at entry to prenatal care. After controlling for gestation at entry and the time in pregnancy when the samples were drawn, there was a significant relationship between dietary folate intake and serum folate at week 28 ($r = 0.17$). Low folate intake (<240 μg/d) was associated with a greater than threefold increase in risk of low birth weight and preterm delivery, after controlling for maternal age, smoking, other factors (including energy), and other nutrients (zinc, fiber, and vitamin B_{12}). Circulating folate at week 28 also was associated with risk; the adjusted odds for low birth weight increased by 1.5% and preterm delivery increased by 1.6% per unit (nmol/L) decrease in serum folate at week 28 (Table 1). Therefore, lower concentrations of serum folate at week 28 were also associated with a greater risk of preterm delivery and low birth weight.

Randomized studies of routine folate supplementation in combination with iron suggested increased maternal hemoglobin, greater gestational weight gain, and an increase in mean gestation duration (Table 1). Fleming *(83)* enrolled 146 Australian women before midpregnancy (week 20) into a randomized trial that included folic acid at a high and a low dose (5 and 0.5 mg/d of folic acid, respectively) as two of the study arms. Eighty-nine women completed the study, with sample sizes of 15 to 20 per group. There were no significant differences in placental weight, birth weight, or gestation duration among the groups. Small sample size and poor statistical power would limit the ability to detect an effect of folate, if any.

Fletcher *(84)* conducted a randomized controlled study comparing iron (200 mg of ferrous sulphate) to iron with folate (5 mg). Supplements were administered to 643 women in London at entry to care. No significant differences were found in birth weight, gestation, or the incidence of congenital defects.

Giles *(85)* performed a double-blind, randomized controlled trial of 692 women from Melbourne, Australia, who were assigned to folate (5 mg/d) or placebo. There were small differences in birth weight and gestation between the groups. However, none were statistically significant.

Blot and colleagues *(86)* assigned 200 French women to iron (105 mg of elemental iron) alone or in combination with folic acid (350 μg/d). Of these, 109 were re-evaluated at delivery. Women treated with folate had higher serum and red cell folate at delivery. The main effect of folic acid was to extend gestation by nearly 1 wk; gestation duration was increased among the folate-supplemented women (40.7 ± 1.2 vs 39.9 ± 1.2 wk), as was birth weight (3461 ± 430 g vs 3303 ± 375 g) and placental weight (660 ± 130 g vs 604 ± 115 g). Tscherina and colleagues *(87)* also found that shorter gestation correlated with lower serum and red cell folate.

A meta-analysis of existing supplementation studies *(88)* recommended further study of the effect of folate during pregnancy on reducing the rate of preterm delivery and low birth weight as an "urgent priority." Many of the trials demonstrated a beneficial effect when a folate supplement was used in combination with iron during pregnancy.

7.3. Homocysteine

An elevation of homocysteine is a metabolic effect of folate deficiency. Hyperhomocysteinemia can occur when dietary folate intake is low. In other cases,

genetic factors and the interaction between genes and the environment may increase the metabolic requirement for folate and the risk of spontaneous abortion, preterm delivery, and other untoward gestational events. This may include many women with medically indicated preterm delivery caused by serious complications in which higher levels of homocysteine have been reported *(89)*.

Homocysteine levels from more than 5000 Norweigan women were linked to previous data in birth registries *(90)*. Higher maternal homocysteine correlated with older age at delivery, higher cholesterol, less multivitamin usage, lifestyle factors (cigarette smoking and high coffee consumption), and a reproductive history that included pre-eclampsia and preterm delivery. In China, preconceptional levels of homocysteine were associated with a substantially increased risk of preterm delivery, as were low levels of the other B vitamins (B_6, B_{12}), whereas folate was not. *(91)*.

Malinow *(92)* assessed the influence of maternal homocysteine and serum folate at term (37–42 wk) on infant birth weight and gestation duration in 35 healthy nulliparas. They reported that higher maternal homocysteine was associated with significantly lower infant birth weight and shorter gestation, whereas higher maternal folate correlated with increased birth weight (univariate analyses). A concentration gradient was detected, suggesting fetal uptake of maternal homocysteine.

In summary, although observational studies of folate and pregnancy suggest a potential benefit of folate supplementation during pregnancy (i.e., a decrease in serious complications and an improvement in birth weight and gestation), randomized trials indicate that routine folate supplementation is not uniformly beneficial. Some individuals at risk because of common genetic polymorphisms that alter folate metabolism or because of environmental factors associated with diet appear to benefit most. Homocysteine may prove to be a more sensitive indicator of risk of low birth weight than either serum or red cell folate.

8. OTHER NUTRIENTS

8.1. Calcium

Another element that has received attention for its possible association with preterm delivery is the macromineral calcium. During pregnancy, there is an increased physiological demand for calcium. A full-term infant accretes about 30 g of calcium, primarily in the third trimester when the fetal skeleton is actively ossifying; to meet these needs, there is enhanced absorption of calcium from the gut *(93,94)*. Diets low in calcium both in general and particularly during pregnancy have been associated with increased blood pressure levels, because smooth muscle reactivity is heightened. During pregnancy, this results in an increased risk of PIH, and thus, hypothetically, leads to preterm delivery. Calcium supplementation trials during pregnancy have been shown to lower blood pressure levels *(95,96)*.

Maternal growth and pregnancy often coincide, and approximately half of all pregnant teenagers continue to grow during pregnancy *(97)*. Bone ultrasound measures of the os calcis during pregnancy have shown greater loss for growing teenage gravidas (–5.5%) than for mature women (–1.9%) *(98)*. Therefore, in the case of an adolescent pregnancy, calcium may be limited by maternal diet but simultaneously driven by the need to retain enough calcium to mineralize two skeletons.

Two calcium supplementation trials among high-risk women (women with very low intakes in Quito, Ecuador and teenagers in Baltimore) showed promising results in

decreasing the incidence of preterm delivery (Table 1). In Ecuador *(99,100)*, length of gestation was increased from 37.4 ± 2.3 wk for the placebo group (n = 34) to 39.2 ± 1.2 wk ($p < 0.01$) for the calcium-supplemented group (n = 22). Among teenagers (age: 16 yr) in Baltimore *(101)*, with similar overall dietary calcium intakes, the calcium-supplemented group had a lower incidence of preterm delivery (7.4%) compared with the placebo group (21.1%; $p < 0.007$). Furthermore, life-table analysis demonstrated an overall shift to a higher gestational age in the calcium-supplemented group (Table 1). On the other hand, a large calcium supplementation trial of more than 1000 adult women from Argentina showed the expected decrease in the incidence of PIH but no effect on preterm delivery *(96)*.

A meta-analysis *(102)* of 14 randomized controlled trials of calcium supplementation involving several thousand gravidas showed significant reductions in systolic and diastolic blood pressure with the administration of calcium salts. Risk of pre-eclampsia was reduced more than twofold among calcium-supplemented women in these trials. An examination of the studies indicated that only two of nine examined yielded statistically significant findings. However, Bucher's analysis yielded no effect of calcium intake on preterm delivery or fetal growth restriction (Table 1 *[102]*).

A large, randomized, double-blind, placebo-controlled trial studied more than 5000 low-risk nulliparous women who were enrolled as patients at medical centers throughout the United States after a prescreen for compliance with supplement use *(103)*. Women were supplemented with 2000 mg/d of elemental calcium or placebo from before midpregnancy until they delivered. Calcium did not reduce risk of pre-eclampsia, PIH, or blood pressure elevation. Similarly, calcium had no effect on obstetrical outcomes, including preterm delivery or infant birth weight. However, it should be noted that the women recruited in this trial had calcium intakes that were atypically high, averaging 1100 mg/d before supplementation.

Recently, a trial of calcium supplementation in Australian gravidas (n = 456) with apparently adequate dietary calcium intakes (median: 1100–1200 g/d) and other characteristics very similar to those in the US study *(103)* reported a positive effect *(104)*. Calcium supplementation resulted in a greater than twofold reduction in the risk of pre-eclampsia and a reduction in the risk of preterm delivery from 10% (placebo) to 4.4% (calcium supplemented) as well as a reduction in low birth weight *(105)*. There also were reductions in admissions for threatened preterm labor (p = 0.03) and preterm PROM (p = 0.08).

The settings in which dietary calcium may reduce risk of preterm delivery are unclear. It is possible that the ability of supplemental calcium to decrease risk may be confined to high-risk populations, where there is either a dietary restriction of calcium intake or, as in the case of adolescents, there is an increased demand for calcium both to meet the needs of the growing fetus and the mother herself.

8.2. n-3 Fatty Acids

Consumption of marine foods that are rich in n-3 fatty acids is also associated with a longer gestation duration. Ecological data from the Faroe Islands, where 50% of the diet is derived from marine sources, show that island women bear infants with substantially higher birth weights (about 200 g higher) and longer gestations than Danes *(105)*. This difference in gestation was hypothesized to arise from a reduction in prostaglandin F_2 and E_2 production or an increase in prostacyclin production with high consumption of n-3 fatty acids.

The clinical trial conducted by the People's League of Health *(106)* in 5022 women in London during 1938–1939 suggested that supplementing the usual diet of British women with minerals, vitamins, and halibut oil extended gestation. Fewer infants were born to the supplemented women before the 40th wk of gestation (20% among supplemented women were born before week 40 vs 24% among the unsupplemented), although there was no difference in mean birth weight.

A trial *(107)* of 533 Danish women who received fish oil, olive oil, or no supplement by week 30 of gestation showed that women taking the fish oil supplement had longer average gestations (4 d) and bore infants with higher average weights (+107 g), which was mostly attributable to the change in gestation. The effect of fish oil was strongest for women with low fish consumption at entry and amounted to an increase of 7.4 d in this group. There was little effect of the supplement on women with high consumption at entry (–1.6 d; Table 1). Thus, the hypothesized effect of n-3 fatty acids on gestation appears to have a threshold, beyond which there is no effect. Furthermore, in this trial, the effect appeared to be specific to the mechanism underlying the initiation of idiopathic preterm labor rather than PROM.

Recently, a multicenter trial of high-risk women enrolled gravidas with a history of preterm delivery, fetal growth restriction, or pre-eclampsia. Women were randomized to either fish oil or olive oil. Fish oil reduced the recurrence risk of preterm delivery approximately twofold but did not alter the recurrence of the other poor pregnancy outcomes *(108)*. A recent prospective and observational study of seafood intake in early pregnancy bolstered this relationship: By week 16 of gestation, risk of preterm delivery was increased nearly fourfold among women with little or no intake of fish *(109)*.

Finally, ecological studies have indicated that the consumption of large quantities of fish during pregnancy is associated with lower blood pressure and thus, in theory, with a lower the incidence of PIH *(110)*. The reduction in the incidence of pre-eclampsia in the People's League of Health Study *(106)* is consistent with this finding. However, because of the multiple supplementation regimen, it could not be attributed to the use of the fish oil *per se*. The recent multicenter trial of fish oil had no effect on the recurrence risk of pre-eclampsia or on maternal blood pressure *(108)*.

8.3. Multivitamin/Mineral Supplements

In the United States, there is limited supplement use by women of reproductive age; overall, about one-fourth of women (26% Caucasian, 15.5% African-American) report taking vitamin or mineral supplements regularly *(111)*. In one study *(79)*, only 16% of low-income Massachusetts gravidas took vitamins before pregnancy. This varied by ethnicity; with Caucasian gravidas (23%) reporting use about twice as frequently as African-Americans (11%) or Hispanics (10%). During pregnancy, 9% of Caucasians, 20% of African-Americans, and 13% of Hispanics used prenatal vitamins erratically (one to three times per week) or not at all. Reasons for noncompliance included maternal confidence that the current diet was good, an unstable home life, side effects attributed to the supplement by the women.

In Camden, 17% of low-income minority gravidas reported using supplements before they got pregnant *(112)*. Periconceptional use was more likely to occur among women with a history of an adverse pregnancy outcome (principally spontaneous abortion in previous pregnancies) and was associated with increased spotting and bleeding during the current pregnancy. Therefore, low-income women appeared to use

supplements when they previously had or currently anticipated a problem with their pregnancies. Based on data from the Maternal Infant Health Survey *(113)*, other predictors of the failure to use vitamin/mineral supplements before or during pregnancy include African-American ethnicity, unmarried status, and age younger than 20 yr with an education below the high school level. In Camden, multiparity and late entry to prenatal care were additional factors associated with supplement nonuse *(112)*.

Information is limited regarding the effects of prenatal multivitamin/mineral supplements on pregnancy outcome. Camden women who use such supplements during pregnancy have reduced risks of preterm delivery (twofold lower than controls) and very preterm delivery (fourfold reduction in risk with first trimester use, twofold reduction with second trimester use). Prenatal supplement use was corroborated by assays of circulating micronutrients. At entry to care, there were no differences among women who went on to use supplements or not, whereas at week 28, circulating levels of ferritin and folate were higher for supplement users *(112)*.

A second observational study *(114)* examined multivitamin use from survey data (the 1988 National Maternal and Infant Health Survey). They reported no effect of multivitamin use on pregnancy outcomes, including preterm delivery; multivitamin use also did not ameliorate the effect of maternal smoking on the fetus. However, these conclusions are limited by the fact that the survey questionnaire was geared toward regular multivitamin use (3 d/wk or more) and did not differentiate between women who used vitamins and those who did not.

Fawzi and colleagues *(115)* examined the effect of supplementing more than 1000 women from Tanzania who were positive for the human immunodeficiency virus with multivitamins, vitamin A, or placebo in a double-blind, placebo-controlled trial. The trial also used a factorial design, which allowed the quantification of the effect of multivitamins with and without vitamin A. Multivitamin supplementation decreased the risk of very preterm delivery by approximately twofold and decreased risk of infant low birth weight and fetal growth restriction.

However, two contemporary clinical trials of multivitamin supplementation (one in Nepal and the other in Mexico) found no effect of multivitamins on preterm delivery. In Nepal, a quasi-experimental study assigned pregnant women by their community to vitamin A plus folic acid; folic acid and iron; folic acid , iron, and zinc; or multiple micronutrients *(116)*. The control group received vitamin A alone. The multivitamin formulation increased birth weight by 64 g and decreased low birth weight by 16%; both changes were statistically significant. The folic acid and iron regimen also decreased low birth weight by 16%, but the increase in infant birth weight (37 g) was not significant. Folic acid without iron had no effect on pregnancy outcome. None of the regimens decreased risk of preterm delivery.

A second randomized study was conducted in Mexico *(117)*. Women were randomly assigned to receive iron with or without multivitamins. Compared to iron alone, multivitamins did not alter birth weight, decrease risk of low birth weight, or decrease risk preterm delivery *(117)*.

8.4. Antioxidants

Pre-eclampsia is a serious complication of pregnancy that is marked by placental insufficiency, the reduced transfer of nutrients from mother to fetus, and an increased risk of fetal growth restriction and preterm delivery. It has been suggested that poor

placental perfusion, secondary to abnormal placental implantation, is the underlying problem and that products from the fetal-placental unit damage endothelial cells *(118)*. In pre-eclampsia, the balance of antioxidant and pro-oxidant forces is disturbed. Concentrations of the products of oxidative stress and lipid peroxidation (such as isoprostanes, malonyldialdehyde and free-radical reaction products) are usually increased and antioxidant activity is usually decreased. Diet may also be a factor in pre-eclampsia. Intake of vitamin C, the first antioxidant to be exhausted by oxidative stress, as well as circulating levels of plasma ascorbic acid are reduced in many women with pre-eclamptic pregnancies *(119)*. Recent clinical trials from England *(120,121)* and India *(122)* suggest that supplementation with high doses of the antioxidants C and E or lycopene from early pregnancy may reduce the risk of pre-eclampsia. In lycopene-supplemented women, fetal growth restriction was reduced 50% ($p < 0.05$) and gestation duration increased by 1–2 wk ($p < 0.05$). In Camden gravidas with adverse pregnancy outcomes (not only those with pre-eclampsia), there was increased oxidative damage to DNA from the maternal—fetal unit early in the third trimester *(123)*. However, all the studies had small samples of women, and further research is needed to confirm and extend their findings.

9. RECOMMENDATIONS

Although the relationship between nutrition and growth—both pre- and postnatal—is well-established, maternal nutrition has emerged as a factor that may increase risk of preterm delivery. Few observational and experimental studies have examined the influence of maternal diet and nutritional status on gestation duration and preterm birth; therefore, for greater confidence, the preliminary positive findings require much more work.

In the developing world, the interaction between nutrition and infection is a well-known cause of childhood malnutrition, morbidity from infectious disease, and death. Similarly, infection/inflammation is an important risk factor for preterm delivery. It is plausible that women who develop infection/inflammation during pregnancy have an impaired immune response associated with subclinical micronutrient deficits; however, there is virtually no research in this important area from either the developed or the developing world *(124)*. Similarly, inflammation may alter the mother's nutritional requirements or lead to low circulating levels of some micronutrients and increased levels of others *(125)*. Thus, it is important to distinguish between a primary and secondary nutrient deficit *(76)*. Again, virtually no research has been done on this topic in pregnant women. In animals, fasting during early or later pregnancy was shown to increase the risk of preterm delivery. In humans, data from the Dutch Famine *(34)* suggested that early starvation increased risk, but fasting or a longer interval between meals increases risk as well *(44,45)*; although the mechanism(s) for this risk is not known, maternal nutrition is clearly implicated.

Populations at high risk of preterm birth appear to have a poor-quality diet, but it is unclear if the maternal diet is a risk factor or a risk marker. For example, among minority groups from the inner cities of the United States, the consumption of fresh fruits and vegetables and whole grains is virtually nonexistent *(126)*. Similarly, diets of women in the lower social class from London failed to meet "...basic maternal needs for a range of nutrients characteristic of whole grain, vegetable and fruit and dairy produce" *(77)*. Vitamin and mineral supplements, an alternative nutrient source, are used more

frequently by the middle classes than the lower classes *(79)*. Therefore, it is prudent to eat a healthful diet that includes fish and to complement the diet with folate-containing multivitamin/mineral supplements both before pregnancy and during its course. Additionally, because a poor diet rarely occurs in isolation, it is also important to seek prenatal care and to maintain a lifestyle free of cigarettes, alcohol, and drugs.

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26 Dietary Polyunsaturated Fatty Acids for Optimal Neurodevelopment

Recommendations for Perinatal Nutrition

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KEY POINTS

- The supply of dietary n-3 and n-6 fatty polyunsaturated fatty acids (PUFAs) affects the composition of plasma and red blood cell membrane lipids of term and preterm infants.
- Human infants require docosahexaenoic acid (DHA) in their diet because they are unable to form this in sufficient quantity from the linoleic acid provided from vegetable oils.
- Dietary n-3 fatty acid deficiency affects eye and brain function of preterm and term infants as measured by electrorelinogram, cortical visual-evoked potentials, and behavioral testing of visual acuity.
- Technological procedures based on chemical and physical separation of the unsaturated fatty acids have permitted the elaboration of nearly pure eicosapentaenoic acid, DHA, and arachidonic acid (AA) for clinical use.
- The development of single-cell oil sources and, more recently, genetically modified plants and animals provides novel forms of long-chain PUFA (LCPUFA) delivery.
- Human milk is the best and only time proven source of fat and essential fatty acids and LCPUFAs in the infant diet.
- Supplementation of formula with both DHA and AA has been demonstrated to support growth and development closer to that of human milk-fed infants without evidence of adverse effects.
- The public health implications of these changes need to be fully evaluated to support this practice on a global scale.

1. INTRODUCTION

Multiple studies over the past four decades have addressed the evaluation of effects of early human malnutrition on central nervous system (CNS) development in experimental

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animals and man. The results of these studies have led to the conclusion that reductions in energy and/or essential nutrient supply during the first stages of life have profound effects on somatic growth and organ structural and functional development, especially for the brain. Malnutrition impairs brain development, leading to reduction of cell replication cycles and dendritic connections. Different regions of the brain are impacted in specific ways: cell number (as measured by DNA content) is especially affected by intra-uterine malnutrition and early postnatal malnutrition, and synaptic connectivity is particularly affected if malnutrition occurs after birth but before the third year of life *(1)*.

After the "brain's growth spurt," alterations in dietary precursors may partially determine neurotransmitter levels (serotonin, norepinephrine, dopamine, acetylcholine) in specific brain regions, and essential and nonessential lipid supply may affect the structural composition of the brain and of myelin sheaths *(2)*.

The link between retarded somatic growth and altered brain development is strong if the nutritional deprivation model is one of early protein-energy malnutrition (PEM). This principle has guided many experimental studies and epidemiological evaluations of the nutrition—mental development relationships. However, there are multiple instances in which somatic growth may proceed unabated while brain structure and functions are significantly altered. The effect of early anemia on brain function does not affect somatic growth; the impact of taurine deficiency on retinal and brain development on nonhuman primates and on human infants is not dependent on structural proteins because this sulfur amino acid is not incorporated in protein synthesis. The role of n-3 fatty acids as structural components of the brain is not associated with effects on growth but, rather, on modifying membrane function and electrical responses. Examples in the opposite direction—namely, of normal mental development and poor growth—are more difficult to find, suggesting that somatic growth is a necessary but insufficient condition to attain normal mental development. Early malnutrition from pyloric stenosis or cystic fibrosis illustrates the capacity of the brain to recover from malnutrition but alone is not sufficient to negate the effect of sustained nutritional deprivation on the developing CNS. The concept that the direct effects of PEM on child development are no longer evident is an important contribution to the relationship between nutritional deficiencies and brain development, because PEM co-exists with other nutritional deficiencies that can also disrupt child development *(3)*.

The study of the influence of specific nutrients on brain development in preterm neonates is in its infancy. Fetal growth retardation is not an isolated nutritional deprivation but, rather, is a combination of many restrictions: oxygen, blood supply, overall nutrients, and various growth factors and hormones. It is likely that better nutritional practices in early life contribute significantly not only to improved survival but also to better growth and development of very premature infants. However, this rather obvious conclusion is not well-substantiated by objective data. One of the few controlled observations in premature infants revealed that head size at age 12 mo was related to the time after birth when very-low-birth-weight infants reached full caloric intake. The relationship between head size and later development in preterm infants is indirect evidence for an effect of better nutrition on developmental indices in later life. Additional supportive evidence can be derived from a study of 526 full-term infants born in Groningen, Holland between 1975 and 1979 and who were followed for 9 yr. There was a significantly lower occurrence of neurological dysfunction in the 135 breast-fed infants (fed for more

than 3 wk) after adjusting for obstetric, perinatal, neonatal, neurological, and social differences *(4)*. Randomized studies by Lucas in preterm infants who were fed controlled diets confirmed the long-term effects on IQ of even just 1 mo of nutritional intervention. Male infants are biologically more vulnerable; they have a lower IQ at age 7 to 8 yr if fed a routine formula as compared to those receiving a nutrient-enriched preparation. Additionally, the risk of cerebral palsy and a verbal IQ less than 85 was lower in the group fed the enriched formula. It is possible that suboptimal nutrition does not permit full CNS recovery after an early insult *(5–7)*. An example of the complexity of this problem involved a study in the late 1940s, which showed that the increase in protein intake required to optimize somatic growth in preterm infants was associated to lower IQ scores in later life *(8)*. The classic Harlem Columbia Study revealed that higher protein intake fed to pregnant women who were at risk of delivering a low-birth-weight infant was associated with increased prevalence of prematurity and to lower developmental indices at 12 mo *(9)*. The research on the effects of energy and caloric intake was reviewed in classic publications *(1–3,10)*. This chapter primarily addresses the role of essential polyunsaturated fatty acids (PUFAs) on neural development in the fetus as well as in preterm and term infants.

2. BACKGROUND ON THE ESSENTIAL POLYUNSATURATED FATTY ACIDS AND THEIR METABOLISM

2.1. Essentiality of Polyunsaturated Fatty Acids

In 1929, George and Mildred Burr *(11)* introduced the concept that specific components of fat may be necessary for the proper growth and development of animals and, possibly, humans . The essential fatty acids (EFAs) were considered of marginal nutritional importance for the human until the 1960s, when clinical signs of EFA deficiency became apparent in infants who were fed skim milk-based formula and in those who were given lipid-free parenteral nutrition. In a clinical and biochemical study of 428 infants who were fed cow milk-based formulations with different types of fat, Hansen *(12)* firmly established that linolenic acid is essential for normal infant nutrition. Daily linolenic acid intake ranged from 10 to 800 mg/kg in the infants, who were fed a fully skimmed milk or a corn/coconut oil-based preparation, respectively. Hansen observed dryness, desquamation and thickening of the skin, and growth faltering as frequent manifestations of linolenic acid deficiency in young infants. More subtle clinical symptoms appear in n-3 EFA deficiency. These include skin changes unresponsive to linolenic acid supplementation, abnormal visual function, and peripheral neuropathy *(13,14)*. The nervous system manifestations of n-3 deficiency are likely caused by an insufficiency of the specific metabolic derivative of linolenic acid (LNA)—namely, docosahexaenoic acid (DHA). Indeed, the high concentrations of DHA in the cerebral cortex and retina suggest its participation in neural and visual function *(15,16)*.

2.2. Long-Chain Polyunsaturated Fatty Acid Metabolic Pathway

The structural role of long-chain polyunsaturated fatty acids (LCPUFAs) derived from EFAs and the functional correlates of specific fatty acids are being increasingly recognized. The LCPUFAs arachidonic acid (AA; 20:4 n-6), eicosapentaenoic acid (EPA; 20:5 n-3), and DHA (22:6 n-3) are important membrane components and precursors of potent bioactive oxygenated products. Eicosanoids (such as prostaglandins, leukotrienes,

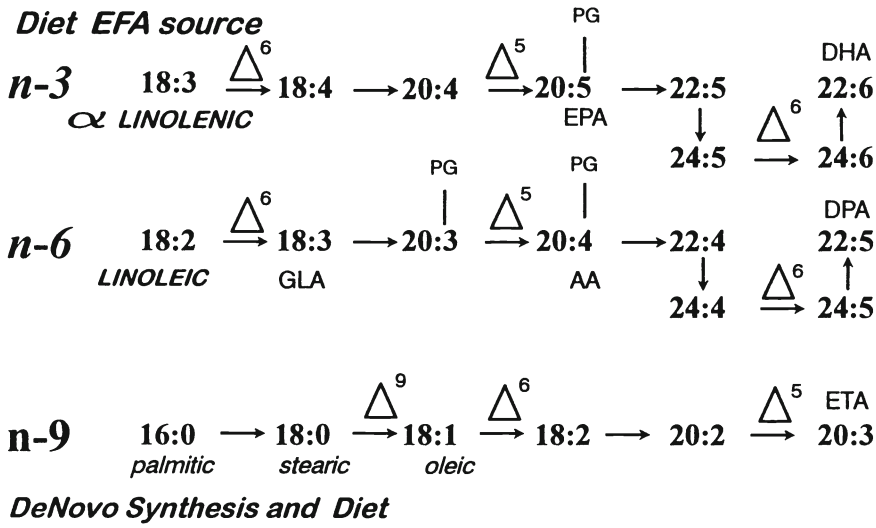

Fig. 1. LCPUFA and EFA metabolism. Parent EFAs are derived from dietary sources for both n-3 (18:3, LNA) and n-6 series (18:2, LA). *De novo* synthesis is able to produce only n-9 LCPUFAs. Elongation occurs two carbons at a time and δ desaturases introduce double bonds at 9,6, and 5 carbons from the carboxylic end of the fatty-acid chain. The final step in the formation of n-3 and n-6 end-products is catalyzed by a peroxisomal β-oxidation. LCPUFAs of interest include 18:3 n-6 (GLA), 20:4 n-6 (AA), 22:5 n-6 (DPA), 20:3 n-9 (ETA), 20:5 n-3 (EPA), and 22:6 n-3 (DHA), EPA, AA, and 20:3 n-6 are immediate precursors of prostaglandins (PG) and other eicosanoids.

and epoxides) derived from AA and EPA modulate or are required in numerous physiological processes. Several clinical correlates associated with deficient or excessive EFA intake have been observed *(17–21)*.

Animal tissues, especially the liver, are capable of further elongating and desaturating the parent EFAs, which generate a family of compounds for the respective families, as shown in Fig. 1. As depicted in the figure, AA can be formed from linolenic acid. AA becomes essential only if the capacity for elongation and desaturation of linolenic acid is limited. This occurs in the cat and other felines. Further details on EFA metabolism can be found in other reviews *(20–23)*. The competitive desaturation of the n-3, n-6, and n-9 series by Δ-6 desaturase is of major significance, because this is the controlling step of the pathway. If n-3 fatty acids are absent or deficient in the diet, the elongation/desaturation of the n-6 compounds generates a significant elevation of docosapentaenoic acid (DPA; 22:5 n-6); if both EFAs are lacking, eicosatrienoic acid (ETA; 20:3 n-9) accumulates *(22,23)*.

The conversion of parent EFAs to LCPUFA is under active regulation; therefore, providing the equivalent amount of linolenic acid or LNA *(24,25)* cannot reproduce the effects of providing AA, EPA, or DHA. The unique biological effects that feeding human milk has on EFA metabolism are based on the direct supply of LCPUFAs bypassing the regulatory step of the δ-6 desaturase. Excess dietary linolenic acid associated with some vegetable oils (particularly safflower, sunflower, and corn oils) may decrease the formation of DHA from LNA and the formation of AA from linolenic acid,

because the δ-6 desaturase is inhibited by excess linolenic acid *(22–25)*. Similarly, excess EPA has an inhibitory effect on δ-5 desaturase activity; this is probably the reason for the lower AA content of membranes that are observed when high EPA marine oil is consumed. Excess linolenic acid (as seen in enterally or parenterally fed infants receiving corn or safflower oil as the predominant fatty acid supply) inhibits the elongation/desaturation of the parent EFAs and thus lowers the LCPUFA supply, which is necessary for membrane synthesis.

2.3. Studies of Essential Fatty Acid Metabolism Using Stable Isotope Precursors

Through the use of stable isotope-labeled precursors, several groups have evaluated the formation of LCPUFA in full-term and preterm neonates *(26–30)*. We administered a single dose of 50 mg/kg of deuterated precursors orally; labeled precursors and products were measured in plasma using negative ion mass spectrometry of pentafluorobenzyl derivatives. Peak concentrations of labeled precursor EFAs were reached on the first day after dosing, and deuterated products increased in concentration with time, reaching peak by 48 h in the term infants and closer to 96 h in the preterm infants. The ratio of concentration of labeled products relative to precursors and indexes of metabolic conversion was approx 1:100 for the n-6 series and 1:40 for the n-3 series *(31)*. There was also evidence for metabolic retroconversion in these studies. The results of all these studies suggest that there is greater biosynthesis of LCPUFA at younger gestational and postnatal ages and indicate a reduced turnover rate for LCPUFA with advancing age. This could be related to the decrease in growth rate and overall metabolic rate during the first year of life *(30)*. These results were limited by the lack of evaluation of tissue pools, especially neural tissue accretion. High activity of δ-6 desaturase in neural tissues suggested a high LCPUFA synthesis in those tissues *(32)*. However, present information on both chemical composition and functional assessment indicates that endogenous synthesis from precursors is insufficient to meet the needs imposed by rapid growth, especially in premature infants.

We have recently characterized formation of LCPUFAs in intra-uterine growth-restricted (IUGR) infants. Our results indicated that after receiving labeled linolenic acid and LNA, there were significantly lower product/precursor ratios in the IUGR group relative to the control groups, which were matched by birth weight and by gestational age. There was a significant decrease in the formation of DHA from LNA for the birth weight-matched control group ($p < 0.01$) but no difference for the gestational age-matched control group. The DHA/LNA ratio was twofold greater in the gestational age controls and threefold higher in the birth weight controls relative to the IUGR infants. The elongation step forming DPA from EPA appeared to be relatively insensitive to birth weight or gestational age, whereas the marked differences in the DHA/DPA ratio indicated that the peroxisomal partial β-oxidation step was clearly affected in IUGR infants. The metabolism of the n-6 LCPUFAs and the various ratios of AA to its precursors did not differ significantly between the IUGR and the two comparison groups. The formation of AA did not require peroxisomal β-oxidation *(33)*. The study of infants who died suddenly as a result of unexplained causes served to document that a strong correlation existed between the composition of the brain cortex and red blood cell (RBC) total lipids and that brain cortex composition was clearly affected by early dietary LCPUFA supply *(34,35)*.

2.4. Effects of Polyunsaturated Fatty Acids on Neural Membrane Function

The dry weight of the human brain is predominantly lipid; 22% of the cerebral cortex and 24% of white matter consist of phospholipids. Studies of several animal species and recent evidence from humans have established that when linolenic acid and LNA or only n-3 fatty acids are deficient in the diet, brain phospholipid AA and DHA decrease, whereas n-9 and n-7 monounsaturated fatty acids and PUFAs increase *(25,34–36)*. Typically, n-3 fatty acid-deficient cells have decreased DHA and increased levels of the end product of n-6 metabolism, DPA. Within the subcellular organelles, synaptosomes and mitochondria seem to be more sensitive to a low dietary n-3 supply as demonstrated by the relative abundance of DHA and the changes in composition of these organelles in response to dietary deprivation.

The functional implications of diet-induced changes in structural lipids have been the subject of much research *(37–39)*. Fatty acid composition of structural membrane lipids can affect membrane function by modifying membrane fluidity, affecting membrane thickness, changing lipid phase properties, or by specifically interacting with membrane proteins *(37–41)*. The changes in neural membranes that are of the greatest potential significance during development are those related to changes in physical properties and changes that affect membrane excitability. Diet-induced changes in structural lipids affect the functional characteristics of excitable membranes in several animal species as well as in human neural cell lines *(42,43)*.

A deficiency of n-3 fatty acids can change membrane physical properties, including membrane-bound enzymes, receptor activity, antigenic recognition, and signal transduction. *(44,45)*. Also, n-3 fatty acid deficiency affects rotational mobility (as measured by fluorescent diphenylhexatriene probes) less than lateral mobility (as measured by pyrene dimer formation within the lipid bilayer) *(46)*. We found that excited dimer (eximer) pyrene formation in human retinal cells, which occurs when two monomers collide, is increased by DHA supplementation of the culture media *(47)*. Membrane composition changes that occur in human retinoblastoma and neuroblastoma cell lines also affect membrane transport mechanisms. A higher DHA content increases the affinity and transport rates for choline and taurine *(48)*. Furthermore, preliminary studies have suggested that the dietary n-3 fatty acid supply may affect nucleotide cyclase activity and rybosylation of guanine nucleotide binding proteins *(50)*.

The role of membrane lipid composition in determining the electrical properties of cultured neuronal cells exposed to exogenous fatty acids has been investigated *(50,51)*. Both n-3 and n-6 fatty acids induced slower rates of rise and, to a lesser extent, lower amplitude of Na^+ action potentials. The reverse effects were observed when saturated or *trans* monoenoic fatty acids were added. It seems likely that these effects were mediated by a change in the number of active Na^+ channels; a change in membrane composition or altered fatty acid availability to the cells may explain these findings *(50)*.

These functions are very important for brain development. LCPUFAs have a role in growth-related events in the neurons and also affect the development of synaptic processes for neural cell interaction. DHA is a major constituent of synaptic end sites, whereas AA is present in growth cones and synaptosomes *(52)*. AA is preferentially released from membrane phospholipids by the action of endogenous phopholipase A2 and participates in signal transduction events that regulate growth cone activity and, ultimately, conversion of growth cones to mature synaptic endings *(53)*. DHA plays a role as a structural component of the membranes—particularly at the synapse site, where the

membrane microenvironment influences neurotransmitter uptake and release *(43)*. AA and DHA also affect the process of interaction between nerve cells during development by activating the transcription of genes that are responsible for brain lipid binding protein. This protein is crucial in a signaling pathway of the response of glial cells to neurons; DHA binding regulates its activity *(54)*. AA is also specifically released by glutamate and *N*-methyl-D-aspartate by stimulating calcium-dependent phopholipase A2. These receptors have been implicated in long-term potentiation, which is considered a likely mechanism for memory and learning *(55)*. Additionally, fatty acids—specifically DHA—affect the expression of genes that regulate cell differentiation and growth of retinal neuronal differentiation. Our recent studies in human fetal retinal explants demonstrated a threefold or greater overexpression for 14% of the nearly 3000 genes that were evaluated when physiological amounts of DHA were added to the serum-free culture media, compared to overexpression in 1% of genes when oleic acid was used *(56)*.

2.5. Sources of Dietary Essential Polyunsaturated Fatty Acids

The principal source of PUFAs for infants is human milk. The amount of PUFAs in human milk depends principally on the mother's diet during pregnancy and lactation and varies according to postpartum age, preterm or term delivery, and maternal diseases that affect lipid metabolism—such as diabetes, cystic fibrosis, and abetalipoproteinemia. AA is the main n-6 PUFA, and DHA is the most important of the n-3 LCPUFA series. The usual ratio of total n-6 to n-3 is 5 to 10:1; however, the ratio may increase to 18:1 if high linoleic acid oils are consumed. The AA/DHA ratio is most commonly 1.5 to 2:1. The variability in LCPUFA in human milk is high and is mainly determined by diet. EPA is found in minimal amounts, the exception being in populations consuming a high fish intake *(57)*. DHA levels range from nearly 0.1% (reported from Germany) to 1.4% (reported in Inuits of North America); however, typical values range from 0.3 to 0.4% *(23,57)*. Makrides reported a longitudinal reduction in breast milk DHA in Australian women on Western diets—from 0.32% in 1981 to 0.21% in 1995 *(58)*. A higher consumption of fish in the maternal diet or DHA supplementation can increase the DHA content of human milk within a short time-frame *(59,60)*.

Vegetable oils derived from corn, safflower, and sunflower predominantly contain linoleic acid; others, such as soy and linseed oils, contain LNA. The latter has higher concentrations of chloroplast membranes, rather than oily seeds, in green leafy vegetables. Thus, products from animals fed in the wild have different compositions of fatty acids than products from grain-fed animals. This is of interest because of the higher DHA content of eggs from range-fed chickens. The introduction of egg yolk as a weaning food in some areas of the world may represent a useful dietary practice to ensure LCPUFA supply in early infancy. Additionally, the use of evening primrose oil or black currant oil provides 18:3 n-6 γ-linolenic acid (GLA), thus bypassing the controlling enzyme, Δ-6 desaturase, which is necessary for AA formation. This approach has been used by some formula manufacturers to improve AA status in young infants.

n-3 PUFAs of marine origin are formed in the chloroplast of the phytoplankton or micro-algae consumed by fish. The main sources for the *de novo* synthesis of n-3 fatty acids are marine autotrophic bacteria, micro-algae, and protozoa, which constitute the zooplankton and phytoplankton. Fish that are higher in the food chain incorporate the n-3 PUFAs and further elongate them to 20 and 22 carbon atom fatty acids containing four, five, and six six double-bonds through the action of specific desaturases.

Table 1
Sources of PUFAs for Use in Infant Formula

	n-*6*	n-*3*
Black current seed	GLA	
Borage oil	GLA	
Egg phospholipids	AA	DHA
Evening primrose oil	GLA	
Fish oil		EPA/DHA
Marine oil fractions		DHA
Animal phospholipids	AA	DHA
Single cell oil	AA	DHA
Transgenic plants/animals	AA	DHA

Therefore, fish concentrate EPA and DHA as triglycerides, mainly in the adipose tissue and in the fat of muscle and visceral organs. The higher the fat content of fish, the higher its content of n-3 fatty acids. Some marine mammals such as seals and polar bears, which feed predominantly on fish, also accumulate relatively high quantities of n-3 fatty acids in their adipose tissue *(61,62)*. Another important source of LCPUFAs is egg yolk phospholipids. The concentrations of PUFAs are different depending on the feed given; the ample use of fish meal in chicken feed has increased egg yolk DHA *(63)*. LCPUFA products for blending in infant formulas can be successfully produced if chicken feed is carefully monitored and refined lipid extraction procedures are used. Egg yolk is currently an important LCPUFA source used in European infant formulas.

Bacterial strains and micro-algae isolated from the intestinal content of some fish show a remarkably high content of EPA and DHA; therefore, efforts have been made to grow these microorganisms in natural or artificial sea water to obtain EPA and DHA for nutritional or pharmacological use. Additionally, selected fungal strains produce concentrated AA that is suitable for human consumption *(64,65)*. The industrial production of AA, EPA, and DHA from strains of these single-cell organisms has been successful; however, the expanded use of these acids depends on price and demand for them relative to the concentrates obtained from marine oils. Single-cell oils provide a safe and abundant source of LCPUFAs, and their mass production has become commercially profitable *(65)*. Very recently, n-3 desaturase transgenic mice (obtained by inserting the n-3 desaturase [fat-1] gene from the invertebrate *Caenorhabditi elegans* in mice) were used to illustrate the potential modification of n-3 content of animal tissues and milk independent of the diet. This offered a novel alternative to change n-3 fatty acid intake by genetically modifying the foods consumed without altering the selection of foods consumed *(66)*. Table 1 summarizes the main sources of LCPUFAs for use in supplementation.

The question of how much LCPUFA to provide to infants is not answered simply by deciding how much breast-fed babies receive in the upper, lower, or the middle point of LCPUFA content of human milk. Rather, the amount must be based on functional response to a given level of DHA. If the effort is primarily focused on demonstrating efficacy, then selecting a value in the upper range is preferable. On the other hand, if serious safety concerns were an issue, selecting a value in the lower range would be more appropriate. Supplementation of formula-fed preterm infants with graded levels of AA (0–1.1% of total fat) and DHA (0–0.76% of total fat) compared to supplementation of

Table 2
Formula With LCPUFAs

	% Total fatty acids	
	n-6	*n-3*
Preterm Infants		
Aletemil. PreNidal Beba (Nestle)	0.5 GLA 0.1 AA	0.1 EPA 0.5 DHA
Nutriprem Cow & Gate (Nutricia)	0.6 GLA	0.2 EPA 0.3 DHA
Prematil (Milupa)	0.2 GLA 0.4 AA	0.2 DHA
Humana OF + (Humana)	0.2 AA	0.2 DHA
Nenatal (Nutricia)	0.6 AA	0.4 DHA
Premilon (Nutricia)	0.45 AA	0.3 DHA
Enfamil Premature LIPIL (Mead Johnson)	0.64 AA	0.32 DHA
Similac Special Care (Abbott)	0.40 AA	0.25 DHA
Term Infants		
Alete PreBeba	0.5 GLA	0.1 EPA
Beba HA (Nestle)	0.1 AA	0.5 DHA
PreAptamil (Milupa)	0.2 GLA 0.4 AA	0.2 DHA
Adapta 90 Inicio (Wander)		0.2 EPA 0.2 DHA
Similac Advance (Abott)	0.4 AA	0.15 DHA
Enfalmil LIPIL (Mead Johnson)	0.64 AA	0.32 DHA
Good Start (Nestle, USA)	0.64 AA	0.32 DHA

human milk-fed babies demonstrated that plasma and RBC LCPUFA levels could be mimicked by supplementing formula at levels close to 0.5% AA and 0.3% DHA *(59,67)*.

3. PUFA IN HUMAN GROWTH AND DEVELOPMENT

3.1. Significance of PUFAs During Fetal Development

The fetus and the placenta are fully dependent on the maternal EFA supply for their growth and development. The major fat deposition in the human fetus occurs during the third trimester, but key phospholipids in placental vessels and uterine vasculature are dependent on EFA supplied by the mother for eicosanoid formation from the moment of conception *(68,69)*.

Maternal dietary linoleic acid and LNA supply serve as precursors for n-3 and n-6 LCPUFA synthesis by the maternal liver. The placental transfer of fatty acids is partially regulated by the transplacental fatty acid gradient. Serum albumin concentration and α-fetoprotein have a high binding affinity for free fatty acids and, therefore, may be important for placental fatty acid transfer *(70,71)*. Mammalian fetuin is a placental protein with a 50-fold greater efficiency in binding fatty acids compared to albumin. Lipoprotein lipase on the maternal surface of the sincytiotrophoblast hydrolyzes maternal triacylglicerol, releasing free fatty acids. Fetal erythrocytes also appear to perform a

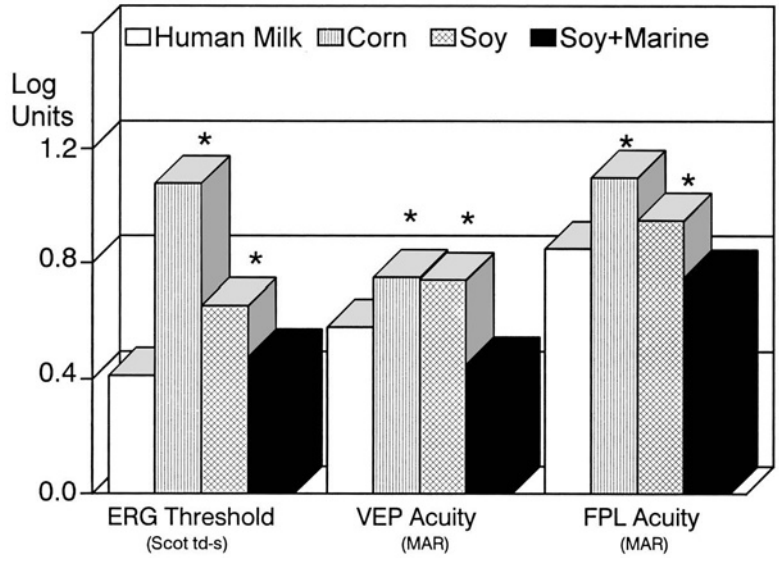

Fig. 2. Rod electrorelinogram (ERG) threshold at 36 wk postconceptional age and visual evoked potential (VEP) and forced-choice preferential looking (FPL) visual acuities of preterm study infants at 57 wk postconceptional age. Rod ERG threshold is the light intensity measured in scotopic troland-seconds (Scot dt-s) required to induce a b-wave amplitude of 2 μV; higher thresholds are associated with lower photoreceptor sensitivity. Larger log minimum angle of resolution (MAR) visual acuity values are associated with poorer visual acuity. Asterisks within a functional measure indicate significantly different from human milk mean values using ANOVA. For experimental details and data *see* refs. *67,68*.

significant role in the placental DHA transfer *(71)*. There is a progressive enrichment in the concentration of AA and DHA in circulating lipids in the fetus during the third trimester—a time when fetal demands for vascular and especially neural growth are greatest *(72)*. Significant increases have been observed in the AA and DHA content of fetal brain tissue during the last trimester of gestation and initial postnatal months *(73)*. A total of 600 g of EFAs are transferred from mother to fetus during a full-term gestation; net uptake approximates 2.2 g per day. AA and DHA are supplied to the fetus from the maternal diet and by endogenous fetal biosynthesis (liver desaturation/elongation).

Studies conducted in different populations that use similar methodologies have shown that the pattern of change in fatty acid composition during gestation and pregnancy is similar among different ethnic/diet groups *(74)*. Populations with higher maternal plasma concentrations of n-6 fatty acids have lower n-3 content and vice versa. DHA concentrations decrease during late gestation, unless the mother's diet is very high in DHA. n-6 cord levels in full-term infants are less dependent on maternal levels than cord DHA. Most n-3 fatty acids, which come into the fetal circulation, are accrued by the fetus despite low maternal n-3 concentrations. These results suggest a need for adequate dietary LCPUFA during pregnancy. This may be particularly important for populations with low n-3 EFA intake, for multiparous women, or during multiple pregnancies *(69,75)*. The changes in maternal fatty acid plasma and RBC content are paralleled by changes in fetal levels measured by cordocentesis. The latter are similar to values obtained at birth, with the exception that a

significantly higher cord linolenic acid level is measured during centesis *(72)*. The results confirm that fetal DHA relative blood content and absolute concentration increase with gestational age. In the case of AA, levels increase or decrease during gestation depending on the study, although there is a better positive correlation with birth weight than that observed with DHA *(76,77)*. Differences in fatty acid profiles at 34 wk of gestation in women delivering preterm full-term babies have shown that RBC and plasma AA are higher in mothers delivering preterm babies. The comparison suggests that maternal AA mobilization and availability may be altered in women who deliver preterm. Preterm maternal n-3/n-6 ratios suggest that perinatal n-3 EFA metabolism plays an important role in preterm birth *(78)*. The fatty acid profile of pre-eclamptic pregnant women shows a lower unsaturation index and is particularly low in n-6 LCPUFAs relative to controls *(79)*.

The PUFA status in pregnancy is of special significance because prostaglandins play a key role in the onset of labor and also induce abortion and labor. AA in amniotic fluid increases at the initial process of human parturition. The use of marine oil supplementation has been proposed in the prevention of pre-eclampsia because maternal blood pressure responses are dependent on AA/EPA balance in the vessel wall. EPA-derived prostanoids oppose the action of AA derivatives; namely, EPA has vasodilatory effects and decreases rather than enhances platelet aggregation. Epidemiological studies and a randomized controlled trial of fish oil supplementation in normal pregnancy demonstrated prolonged gestation with higher birth weight in the group supplemented with n-3 *(80)*. Despite the lack of efficacy of fish oil supplements given during the second trimester of gestation in reducing the risk of pre-eclampsia, IUGR, and preterm labor, the potential benefit of supplementation given periconceptionally remains open *(81)*. Recent data from Norway indicate that there may be important benefits for children when mothers are supplemented with LCPUFAs. Children born to mothers who had taken cod liver oil during pregnancy and lactation scored 4 points higher on the Mental Processing Composite of the K-ABC at age 4 yr compared to children whose mothers had taken corn oil. Furthermore, the children's mental processing scores at age 4 yr correlated significantly with maternal intake of EPA and DHA during pregnancy. In a multiple regression model, maternal intake of DHA during pregnancy was the only variable of statistical significance for the children's mental processing scores at age 4 yr *(82)*.

3.2. Significance of Polyunsaturated Fatty Acids for Preterm Infants

The brain growth and LCPUFA deposits during the last trimester of pregnancy suggests that preterm infants may be especially vulnerable to insufficient access to EFAs. Preterm infants show a progressive decrease in LCPUFAs in plasma and RBC lipids, especially if the early diet lacks LCPUFAs. Human milk-fed infants and infants receiving DHA and AA in formula had higher levels in plasma, RBCs, and buccal mucosal cells than infants fed DHA- and AA-free formula *(83–87)*.

3.2.1. Controlled Studies in Preterm Infants

We studied preterm infants who received human milk or were randomly assigned to formulas with different EFA contents from 30 wk of gestation to early infancy. The functional impact of n-3 fatty acid supplementation included enhanced retinal maturation of the rod photoreceptor responses in supplemented infants, which mimicked matched human milk-fed preterm infants of similar postconceptional age (36 wk). By 57 wk (a time when retinal development is nearly complete), the difference in photoreceptor

function was not apparent, with the exception of changes in oscillatory potentials, which reflect inner retinal signal processing. Visual acuity tests throughout the 6-mo study also were less mature in infants who received formula that was devoid of DHA, despite ample provision of LNA. The n-3 LCPUFA-supplemented group had significantly better visual acuity (as measured by visually evoked potential [VEP] and forced-choice preferential looking [FPL]) than the control group. Highly significant correlations were found for both VEP and FLP visual acuity when compared to the level of DHA in multiple lipid fractions from study infants *(88–90)*. Our more recent studies conducted in Chile demonstrated no LCPUFA effect on auditory brainstem-evoked responses, which is in agreement with the study by Faldella *(91)*. There was an increased maturation of the sleep–wake cycle regarding less indeterminate sleep and more quiet (non-rapid eye movement) sleep in the human milk-fed group compared with the randomized formula-fed groups. At 18 mo, the Bayley scale demonstrated better developmental indices in the human milk-fed group but no differences within formula-fed groups. The findings in developmental indices suggest that environmental influences may have a greater effect than the early diet.

Carlson's randomized clinical study of preterm infants who were supplemented with LCPUFA demonstrated better visual acuity using behavioral measures in infants up to age 4 mo. After this time, control infants "caught-up" in visual function measures. These investigators also reported evidence of more rapid visual processing, as measured by the Fagan test of visual recognition at age 6 to 12 mo in LCPUFA-supplemented infants *(92,93)*. The reduction in AA when fish oil was provided as a source of n-3 fatty acids was associated with reduced weight and growth in length (94). In a second preterm infant study that used low EPA marine oil for up to 2 mo corrected age, Carlson demonstrated improved visual development at the 2-mo follow-up and a 10-point IQ difference that favored the DHA-supplemented group at 12 mo *(95–97)*. No significant drop in AA or deleterious effects on growth was observed when low EPA marine oil was used. The DHA-supplemented group had shorter look-times in the novelty preference test at 9 mo, which is suggestive of better visual processing.

The largest, collaborative, multicenter study of a large group of preterm infants included 450 preterm infants fed LCPUFA formula that was supplemented with different AA and DHA sources: fish oil and egg phospholipids or fungal oil. The level of DHA was 0.25% of total fat in preterm formulas and 0.15% in follow-up formula; both formulas contained 0.4% AA. Significant differences were found in sweep VEP at 6 mo, favoring the LCPUFA-supplemented formula group rather than the control formula group. No differences were found in behavioral test of visual acuity (Teller cards) or in Fagan habituation or Macarthur vocabulary tests. An advantage in the Bayley score was observed at 12 mo for infants who weighed less than 1250 g at birth in the LCPUFA-supplemented group. There were no consistent differences in weight, length, head circumference, anthropometric gains, or preterm morbidity *(98)*. Neither long-term follow-up of these infants nor other studies have been reported.

3.3. Significance of Polyunsaturated Fatty Acids for Full-Term Infants

Similar questions to those addressed in the previous section on low-birth-weight infants have been posed for healthy full-term infants. The finding of lower plasma DHA concentrations in infants who were fed formula compared to breast-fed infants suggests that present formulas provide insufficient LNA or that chain elongation/desaturation enzymes are not sufficiently active during early life to support tissue accretion of DHA. Furthermore,

necropsy studies in infants born at full-term who died from Sudden Infant Death Syndrome revealed that brain composition is affected by type of feeding. Human milk feeding resulted in higher DHA content in the brain cortex than cow milk-based formula feeding *(34,36,99)*. The first controlled randomized study compared infants fed a formula that was supplemented with 0.36% DHA, a formula providing ample LNA but no DHA, and a breast-fed reference group. This study revealed delayed visual acuity at ages 4 and 6 mo in the group taking formula that lacked DHA, relative to those receiving formula that was supplemented with LNA plus DHA; infants receiving formula that was supplemented with LNA and DHA displayed results similar to the breast-fed control group *(100)*.

Studies by Innis *(101–103)* concluded that the need for DHA supplementation should not be based on the alleged improved visual acuity of breast-fed infants, because despite differences in DHA content in blood, no differences were found in preferential looking acuity between breast-fed and unsupplemented formula-fed infants. The formula used in this study contained 2.1% of total fat as LNA but no LCPUFAs. On the other hand, a multicenter trial using formula supplemented with 0.2% DHA and 0.4% AA did not show an effect of this level of supplementation on visual acuity or growth *(104)*. A study with different levels of LNA (0.4 to 3.2% of total lipids) in full-term infants did not show an effect on transient visual-evoked responses at ages 120 and 240 d *(105)*. In a small trial with LCPUFA-supplemented formula-fed and breast-fed infants, the visual acuity (measured by swept steady-state visual-evoked potential) was different. The LCPUFA-supplemented formula-fed group had better results than groups receiving standard formula and worse results than breast-fed infants *(106)*.

A beneficial effect of DHA, AA, and GLA supplementation on psychomotor development has been reported at ages 4 and 12 mo, but not at 24 mo, as assessed by the Brunet-Lezine method *(107,108)*. The authors reported a strong association between the erythrocyte phosphatidylcholine arachidonic/linoleic acid ratio and developmental quotient at age 24 mo, but there was no relationship with the dietary intervention during the first 4 mo of life *(109)*.

A randomized trial in full-term infants who were fed formula that contained no LCPUFA or formula that contained DHA or AA plus DHA found no differences on Bayley Scale scores at 12 mo but found lower language comprehension and production scores measured on the MacArthur Communicative Development Inventory in the DHA-supplemented group compared to the AA plus DHA-supplemented group *(110)*. These children have been followed to age 3 yr and have not demonstrated differences on psychomotor development and vocabulary *(111)*.Willats studied 44 full-term infants who were fed formula supplemented with DHA and AA or formula with no LCPUFAs during the first 4 mo of life. Infant cognitive behavior was assessed at age 10 mo by a means-end problem-solving test. The supplemented group had significantly more intentional solutions and scores than infants who were fed non-LCPUFA formula. Higher problem-solving scores in infancy are related to higher childhood IQ scores *(112)*. In an interesting model that studied the effects of DHA present in human milk, Gibson supplemented mothers to produce breast milk with DHA concentration that ranged from 0.1 to 1.7% of total fatty acids. Infants' plasma and erythrocytes phospholipids were related to breast milk DHA in a saturable manner. Specifically, there was no further increase in breast milk DHA above 0.8% of total fatty acids. At 12 mo, the developmental quotient had a low but significant correlation with erythrocyte DHA. This correlation was not evident at 24 mo *(58)*. Innis et al. *(113)* addressed whether the natural variability in human milk DHA is important for

visual and neural development in infants. Visual acuity at ages 2 and 12 mo, but not 4 and 6 mo, was significantly related to blood lipid DHA at 2 mo. No relationship was found between infant AA or DHA status and scores on the Bayley Developmental indexes or novelty preferences; however, LCPUFA was related to language production and comprehension assessed at ages 14 and 18 mo *(113)*. In a 3-yr follow-up of visual acuity maturation study, we found that formula-fed full-term infants had lower operant preferential looking (OPL) than human milk-fed infants *(114)*. The cohorts were breast-fed from birth to at least age 4 mo or fed for 12 mo with formula that contained ample linolenic acid and 0.5% of the total fat as LNA. The breast-fed group was weaned to an oleic acid (18:1)-predominant formula and received egg yolk through age 12 mo. The breast-fed group maintained higher plasma and RBC membrane phospholipid DHA concentrations throughout the first year of life. At age 3 yr, stereo acuity, as measured by OPL techniques, was more mature in the breast-fed infants compared to the formula-fed group; 92% of the breast-fed group had mature OPL stereo acuity, whereas 35% of the infants in the formula-fed group met the maturity criteria. Visual recognition in the breast-fed group was also better: whereas only 61 % of the formula-fed infants had a perfect score, 93% of the breast-fed group had a perfect score *(114)*.

Human milk provides a unique nutrient mix, hormones, and special growth-promoting factors; these, along with LCPUFAs, may play a role in CNS development. Other observational studies of breast-fed and formula-fed infants in relation to cognitive development have been published since 1929 *(115–124)*. A long-term effect of early feeding on brain development was suggested by results from the controlled trial of preterm infants, which indicated that feeding human milk that contained LCPUFAs by nasogastric tube for 28 d was associated with an 8.3-point increase in IQ at age 8 yr compared to a formula-fed group (which was devoid of LCPUFAs), after controlling for socioeconomic and other maternal variables *(5)*. These controlled observations in preterm infants indicate that human milk may offer unique advantages for brain development. With the exception of this study, which was performed with human milk by tube feeding, all other studies have been performed with nonrandomized breast-fed infants. On average, mothers who succeed at breastfeeding are different from those who use formula: they have a higher socioeconomic status, better educational level and educational achievement, higher intelligence, less symptoms indicative of maternal depression, and greater preoccupation with infant development. The act of breastfeeding provides a mode of mother–infant interaction, contributes to bonding, and may enhance cognitive development. The most recent study using extensive covariate adjustment showed that the benefit of breastfeeding on cognitive development at age 11 yr was no longer significant after maternal IQ and home environment were incorporated into the analysis *(125)*.

Results from studies in Dallas indicated a persistent benefit on visual acuity development for the first year of life in DHA-supplemented formula-fed infants compared with infants who were fed formula that had ample LNA but no LCPUFA *(126)*. The dietary effects on visual acuity development were evident using sweep VEP acuities but were absent if the FPL behavioral measure of acuity was used. The differences were significant during the periods of rapid changes in development of VEP acuity, (i.e., in the first 20 wk and near age 12 mo). The developmental outcome of these infants also showed important differences *(127)*. Scores on the Bayley Mental Development Index II at age 18 mo for the DHA plus AA group were significantly better than those observed in the non-LCPUFA formula-fed infants. A 7-point difference in normalized

mental development index score was highly significant, despite the relatively small sample size ($n = 20$ per group). The small variability in developmental score obtained likely resulted from the highly homogenous population studied and the fact that one observer evaluated mental development in all subjects. The DHA-only group had marginally higher mental development index scores than controls. This was the first randomized controlled study that reported an effect of LCPUFA on mental development at age 18 mo. Moreover, positive significant correlations were noted between blood DHA levels at 4 mo with measures of visual acuity at 1 yr and mental development at 18 mo. The existence of a relationship between early biochemical and later functional data suggests that visual function and neurodevelopment may be related but do not constitute proof of causality. Using LCPUFA-enriched and control formulas, Makrides et al. *(128)* did not find diet-induced differences in VEP acuity or Bayley Mental Development Index scores during the first year of life. In this case, breast-fed infants had higher Mental Development Index scores than formula-fed infants at age 2 yr, even after adjusting for environmental variables *(128)*.

In one of the largest controlled full-term infant studies to date, Lucas et al. *(129)* did not find a beneficial effect of LCPUFA supplementation on cognitive development in a group of 309 infants who were randomized to formula diets with or without LCPUFA. A reference group of 138 breast-fed infants were included for comparative purposes. No biochemical data on fatty acid composition of plasma or tissues were obtained in this study, which limited the assessment of compliance to the test formula. Follow-up studies at age 18 mo revealed no benefit on cognitive or motor development. No adverse effects of LCPUFA supplementation were noted regarding infection, atopy, or formula tolerance. Unfortunately, the interpretation of these data is limited by the fact that study formulas differed not only by the presence or absence of LCPUFAs but also by the presence or absence of several other fatty acids and key components, such as choline and cholesterol content. The expected higher mental development index score in breast-fed infants compared to standard formula-fed infants was not apparent in this study, and because there was no plasma or tissue biochemical evaluation of infant EFA status, a relationship between LCPUFA status and neurodevelopment could not be established. Additonally, the population studied had a low educational level—that is, close to 20% had no formal education at all, and about 70 % had not completed a high school education. Mean mental development index values for all groups were 4 to 6 points below the norm, suggesting that these groups may not have been representative of the normal population in the United Kingdom but, rather, were a group subjected to an environmental factor that restricted their neurodevelopment.

Austead et al. *(130)* conducted a parallel double-masked, randomized trial of 239 full-term infants who were fed formulas with or without AA and DHA for 1 yr. A group of breast-fed infants ($n = 165$) who were weaned to formulas with and without AA and DHA was also studied as a reference. Infants in the formula groups were randomized before age 10 d to a control formula with no LCPUFAs ($n = 77$) or to one of two otherwise identical formulas that contained AA plus DHA (AA, 0.46% and DHA, 0.14% of total fatty acids) from either egg-derived triglyceride ($n = 80$) or fish oil and fungal oil as a source of LCPUFAs ($n = 82$) at levels similar to the average found in breast milk samples (as measured in the reference group). All formulas contained 50% of energy from fat with the essential dietary fatty acids: linolenic acid (20% fatty acids) and LNA (2% of total fatty acids). The main study outcomes were AA and DHA levels in plasma

and RBCs, and multiple measures of infant development at multiple ages from birth to 14 mo (growth, visual acuity, information processing, general development, language, and temperament). AA and DHA levels in plasma and RBCs were higher in groups supplemented with AA plus DHA than in the control formula group and were comparable to those in the breast-fed reference groups. No developmental test results distinguished these groups. Expected differences were found regarding family demographics associated with breastfeeding, but no advantages to breastfeeding were demonstrated on any of the developmental outcomes. The authors of this large study concluded that the findings did not support adding AA plus DHA to formulas that contained 10% of energy as linolenic acid and 1% of energy as LNA to enhance growth, visual acuity, information processing, general development, language, or temperament in healthy full-term infants during the first 14 mo of life *(130)*.

The results of a meta-analysis conducted regarding the effect of LCPUFAs on visual acuity maturation in full-term infants showed a positive effect for assessments conducted during the first 2 to 4 mo of life; the results at 6 mo were equivocal, and some studies showed a significant beneficial effect at 12 mo. San Giovanni et al. *(131,132)* concluded that there was a significant overall advantage for the LCPUFA-supplemented infants regarding visual acuity. In an attempt to explain the differences in visual acuity responses from the randomized studies, we conducted a "meta-regression" considering the effective DHA supplied by the formula in each study as a key variable to explain visual acuity maturation. This analysis was used to define the role of a DHA dose in establishing the magnitude of the effect on visual acuity; the DHA dose was used as the independent variable, and visual acuity was used as the dependent variable. To date, no controlled study has been conducted regarding dose–response of DHA on visual acuity. However, if we consider that the main dietary determinants of DHA status are the LNA and EPA provided and the preformed DHA consumed, we can attempt to define the DHA-equivalent dose across studies and compare the visual acuity effect. To estimate DHA equivalents from LNA and EPA, we used conversion ratios derived from our stable isotope studies *(31)*. This approach, termed "meta-regression," revealed that visual acuity maturation measured at age 4 mo is positively and strongly related in a linear fashion with the estimated DHA supply expressed as DHA equivalents *(133)*.

The follow-up of phenylketonuric infants treated with early protein restriction may also serve to evaluate the effect of LCPUFA deficiency. The effect of 12 mo of DHA supplementation on functional developmental shows a weak but significantly positive association between plasma LCPUFAs at diagnosis (higher in breast-fed infants) and neurodevelopmental indices through the first year of life *(134)*. Additionally, diseases linked to peroxisomal disorders (where there is an altered peroxisomal β-oxidation necessary to form DHA) provide an opportunity to assess DHA effects on brain development. Supplementation with preformed DHA but not with EPA has been demonstrated to be beneficial, especially if less generalized disease is present *(135)*. The benefit of DHA supplementation in patients with peroxisomal diseases suggests that DHA is also involved in myelogenesis, because there is an improvement of myelination seen in magnetic resonance images after long-term DHA supplementation *(136)*. This could be important in white matter diseases of micropremies. Recent evidence has suggested that the weaning diet has progressively become depleted of n-3 LCPUFAs; therefore, several studies have indicated a potential need for DHA supplementation through age

12 mo for optimal neurodevelopment. Even fully breast-fed infants randomized at the time of weaning (age 4–6 mo) to formula with and without LCPUFA supplementation revealed measurable biochemical and functional effects in terms of RBC DHA content and improved visual acuity maturation at age 12 mo *(137–139)*.

4. CONCLUSIONS

Studies presented in this chapter provide clear evidence that dietary n-3 fatty acid deficiency affects eye and brain function of preterm infants as measured by ERG, cortical visual-evoked potentials, and behavioral testing of visual acuity. Preterm infants require DHA in their diets because they are unable to form these in sufficient quantity from LNA provided by soy oil-based formula products. Dietary n-3 and n-6 fatty PUFAs supply results through discernible differences in the fatty acid composition of plasma and RBC membrane lipids in full-term and preterm infants. Changes in membrane structure likely are responsible for the observed functional effects. The evidence from full-term infants indicates that in several, but not all, studies the DHA-supplemented groups have enhanced visual acuity and, possibly, better cognitive development as compared to nonsupplemented infants. Supplementation with both DHA and AA has been demonstrated to support growth and development closer to that of human milk-fed infants, without evidence of adverse effects. Human milk is the best and only time-proven source of fat and EFAs in the infant diet. Technological procedures based on chemical and physical separation of the unsaturated fatty acids have permitted the elaboration of nearly pure EPA, DHA, and AA for clinical use. The development of single-cell oil sources and, more recently, genetically modified animals has allowed the provision of novel forms of LCPUFA delivery. Before the 1990s, low LNA was found in most infant formulas; currently, virtually all infant formulas in developed countries are supplemented with LNA, and most manufacturers in Europe, Japan, and North America have added either DHA, DHA plus AA, or these fatty acids plus GLA to preterm and full-term formulas as a choice for infant feeding. The public health implications of these changes need to be fully evaluated to support this practice on a global scale.

5. RECOMMENDATIONS FOR FORMULA-FED INFANTS

Exclusively breast-fed infants receive sufficient EFA and LCPUFAs from breast milk during the first 6 mo of life to support normal eye and brain development. Considering that both n-6 and n-3 are essential nutrients for premature and full-term infants, the following recommendations are suggested for formula fed infants:

1. The total EFA requirement (n-6 + n-3) for premature infants should be set at 5 to 6% of total energy, although up to 10 % can be provided safely. This represents approx 0.6 to 0.8 g/kg per day, with an upper limit of 1.5 g/kg.
2. The parent n-6 EFA (linoleic acid) supply should be 0.5 to 0.6 g/kg daily, and because desaturase and elongating enzymatic activity are limited in early life, formulas for infants should provide 60 to 100 mg/kg per day as preformed AA.
3. The total n-3 fatty acid supply should be 70 to 150 mg/kg daily. Because desaturase and elongase enzyme activity are limited in early life, formulas for infants should provide 35 to 70 mg of n-3 LCPUFA per kilogram of body weight per day as DHA.
4. The total linoleic acid supply should not exceed 12% of total energy, because excess linoleic acid may adversely affect the formation of LCPUFAs.

5. The ratio of total n-6 to n-3 fatty acids that is present in the early diet should be maintained within a range of 5:1 to 15:1. The ratio of DHA to AA should be from 1:1 to 1:2, because excess DHA may lower conversion of linoleic acid to AA.
6. After age 6 mo, weaning foods (formula and or solid foods) should contain LCPUFAs to continue to support neurodevelopment.

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27 Micronutrient Deficiencies and Maternal Thinness

First Chain in the Sequence of Nutritional and Health Events in Economic Crises

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KEY POINTS

- There is an important and potentially severe health and nutrition impact resulting from economic crises that are erupting around the globe, which are especially affecting the quality of diets.
- Such impacts can cause a disadvantage for the neuro-intellectual and physical development of an entire generation.
- Surveillance of changes in micronutrient status offers a sensitive means of tracking health and nutrition crises.
- Tracking mothers' body mass index measurements at a population level reflects changes in food consumption better than measurements of changes in underweight children.
- Childhood anemia levels (reflecting iron deficiency) and maternal night blindness (reflecting vitamin A deficiency) are sensitive indicators of a change in intake of micronutrient-rich, more expensive foods.

1. INTRODUCTION

The scale of crises affecting populations has moved from the local level, in hunter–gatherer societies, to subcontinental epidemics and famines (e.g., the "Black Death" in medieval Europe), to world wars. These were clear disasters for those involved, both individuals and nations, and this has been reflected in high mortality levels and, usually, malnutrition. In the latter half of the 20th century, these crises have often become more subtle, such as those caused by differing political and economic systems. It is now well-recognized that political restructurings in the early 1980s had measurable impacts on malnutrition levels and national health, particularly among those

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most vulnerable *(1)*. However, the most recent national economic crises are again different and have devastated the economies and social structures of several Asian societies and countries such as Argentina and the Russian Federation, with repercussions felt throughout an increasingly globalized world. This chapter shows that these crises not only result in an immediate impact in these countries but can also result in a "lost generation" in countries such as Indonesia. Furthermore, using the Indonesian example, this chapter shows that such crises are often best characterized and tracked by using micronutrient and maternal malnutrition, rather than childhood malnutrition, as indicators. We illustrate that these types of crises are felt more strongly among the urban poor than among the rural population. Finally, we discuss the global significance of these findings.

2. POVERTY AND DEVELOPMENT

The second half of the 20th century has been called the "era of development" *(2)*. This era of development coincided with the "era of the Cold War." The two superpowers (the United States, which championed Capitalism, and the USSR which championed State-led Socialism) competed for influence among the newly independent, ex-colonies. Throughout this period, development was mainly applied to one part of the world: the so-called "Third World." In the early 1970s, the Third World developed a nonaligned philosophy based on three basic principles: peaceful co-existence, anti-colonialism and the struggle for economic independence. However, political polarization and economic differentiation in the following years largely destroyed these alliances.

In the 1980s, neoliberalism became the dominant philosophy in (economic) development, and these divisions became even more prominent. During that decade, markets in Latin America were not functioning well, their gross national product declined for 3 yr consecutively, and budget deficits and inflation were very high *(3)*. In 1989, John Williamson coined the term "Washington Consensus" to refer to a set of economic policy prescriptions to Latin American countries, which had emerged by consensus among the US politicians, technocrats of the International Monetary Fund (IMF) and World Bank *(4–6)*. In Williamson's original paper, he summarized this advice in 10 propositions: fiscal discipline; a redirection of public expenditure priorities toward fields offering both high economic returns and the potential to improve income distribution, such as primary health care, primary education, and infrastructure; tax reform; interest rate liberalization; a competitive exchange rate; trade liberalization; liberalization of inflow of foreign direct investment; privatization; deregulation; and secure property rights. These propositions were a reflection of the fact that many economists had become convinced that the key to rapid economic development lay not in a country's natural resources or even in its physical or human capital but, rather, in the economic policies that it pursued. Therefore, the Washington Consensus places economic growth as a predominant part of of its strategy, arguing that it typically promotes human development. The timing of the Washington Consensus coincided with the collapse of the Soviet system and its ideological apparatus.

In 1996, Lester Thurow, a professor of economics and former Dean of MIT's Sloan School of Management wrote about "the future of capitalism." In the summer of 1994, Mexico was the "star pupil" in its pursuit of neoliberal reforms; the budget was balanced, more than 1000 State-owned companies had been privatized, government regulations had been slashed, and tariffs and quotas were dramatically reduced. Private capital was

pouring in. However, by April 1995, Mexico's economy had collapsed; the average purchasing power had declined by 30%, and 500,000 Mexicans had lost their jobs. Mexico had implemented all the policies that economists had recommended, as had Argentina more recently. Clearly, the nature of economic crises was changing.

Thurow borrowed two concepts from the physical sciences: the punctuated equilibrium theory of evolution from biology and plate tectonics from geology. Punctuated equilibrium is the phenomenon in which the dominant species dies out rapidly as the environment suddenly changes. An analogy for this theory might include the economic era of agriculture, which existed for thousands of years and was then replaced by the Industrial Revolution within 100 yr.

Earthquakes and volcanic explosions are a result of the invisible movement of the continental plates beneath the surface of the earth. In his book, Thurow recognized five economic "plates": the end of communism; a technological shift to an era dominated by man-made brainpower industries; a demographic never seen before; a truly global economy; and an era in which there is no dominant economic, political, or military power. Recent events and the current militarism and status of the United States as the one global superpower demonstrate that this presumption, at least, was premature. Nevertheless, he concluded that as the "Second World" collapsed, the Third World had splintered. Within it, there were clear winners (the little tigers of Hong Kong, Singapore, Taiwan, and South Korea), potential winners (Thailand and Malaysia), those rapidly integrating with global Capitalism (China), and the losers (most African countries). The Third World was said to be gone just as the Second World was gone *(7)*.

One year later, in 1997, the Asian economic crisis began. Not only had the predicted economic instability happened earlier than predicted, it also hit hardest among the so-called "clear winners." In the three decades before 1997, the economic development and the reduction of poverty in east Asia was greater than in any other region. However, the region had been successful despite the fact that the countries had not followed most of the dictates of the Washington Consensus. Macro-economic stability, one of the key elements of the success of the east Asian miracle, was brought about by the important role that the government played in east Asia's economies *(8)*. Many observers were surprised that when the Asian economic crisis broke out, the IMF was very critical of the east Asian countries because, not long before, the IMF had forecast strong growth for the region *(9)*.

As expected, the IMF recommended a tight monetary policy, stringent fiscal policy, and structural and banking reforms in exchange for multibillion dollar bailout schemes. Not only were these prescriptions relatively unsuccessful in the short-term, the effects on health (as measured by the impact on malnutrition, among other human factors) were—and, to some extent, continued to be—seriously underestimated *(10)*. As the economic recession spread to other countries of Asia and adjoining regions, fear of a much larger meltdown continued in eastern Europe and Latin America. Even the United States currently carries the greatest financial deficit ever seen globally. It is important that lessons be learned from these events, not only from the political and economic points of view but also regarding how to predict, judge, and track their effects on the health, nutrition, and potential development of populations.

The work of Amartya Sen in the 1980s significantly advanced the notion that human and social development are key aspects in the overall development of nations. These ideas led to the relatively new measure known as the Human Development Index, which was developed by the United Nations Development Programme to describe social

development indicators of countries. A further sign of increasing distrust in the Washington Consensus and its relatively little regard for human capital was demonstrated in the criticism leveled by Joseph Stiglitz regarding the Washington Consensus-based approach of the IMF and its heavy emphasis on painful economic growth policies, which came at the expense of immediate human and social welfare needs in the face of the Asian economic crisis.

In light of Sen's human development paradigm and Stiglitz's technical critique of IMF policies, it is important to determine whether there is hard data to support the idea that human and/or social needs are disrupted by the introduction of stringent economic growth-focused policies and how that possible disruption affects the overall development of an affected country. As demonstrated in Asia, very large numbers of people in countries affected by economic crisis have (or will have) less purchasing power. What will be the impact on the health and nutritional status of these segments of the population? Furthermore, how would an impact on health and nutritional status among these countries' human resources affect its future development?

3. MICRONUTRIENT DEFICIENCIES, HUMAN CAPITAL, AND LOST GENERATION

It is clear from the previous section that there are a growing number of economists who believe that the economic development and well-being of the populations of less developed countries partly depend on their human capital, which includes the level of education received. For several decades, it has been known that micronutrient deficiencies (such as vitamin A deficiency, iodine deficiency, and iron deficiency) are associated with poverty and that these deficiencies have had major effects on populations all over the world, including premature death, poor health, blindness, stunting, mental retardation, learning disabilities, and low work capacity *(11,12)*.

Iron deficiency is one of the most common micronutrient deficiencies in the world. The human body needs iron to produce hemoglobin, the protein in red blood cells responsible for carrying oxygen. Iron is also a component of many enzymes that are essential for the adequate functioning of brain, muscle, and immune system cells *(13)*. Iron deficiency is strongly correlated with poverty, because the main sources of bioavailable iron are animal products, which are relatively more expensive than nonanimal food products.

The economic cost of iron deficiency has only recently been recognized. A review by Horton and Ross *(14)* analyzed the economic cost of iron deficiency anemia (IDA) based on two outcomes. The first outcome is the lower future productivity of children with IDA (based on studies that show long-term reduced cognitive development resulting from IDA among children below age 2 yr). The second outcome is the immediate-term effect of lower productivity among adults who are anemic.

3.1. Lower Future Productivity of Iron-Deficient Children (Lost Generation)

Although the relationships are complex, there is ample scientific evidence to show that IDA is associated with impaired performance in a range of cognitive and physical coordination functions in children of different age groups *(15)*. These functions include mental development, motor coordination development, cognitive (awareness and judgment) abilities, social and emotional development, and school achievement. Evidence from animal studies also indicates that the behavioral and developmental effects of IDA may not be as easy to correct in infants, compared with preschool or school-aged children and adolescents.

Grantham-McGregor and Ani *(16)* concluded that, although the causal relationship was still uncertain, longitudinal studies indicated that children who were anemic in infancy continued to have poor cognitive and motor development and school performance into middle childhood. Observational studies found similar results among older children, but treatment studies with iron were more effective in this age group.

Another important consequence of iron deficiency is an apparent increased risk of lead poisoning in children. Children who are iron deficient have a greater susceptibility to toxicity from heavy metals, including lead *(17)*. Lead is very toxic during the fetal stage of life as well as during early childhood and causes long-term damage to the nervous system *(18–20)*. In urban areas in developing countries, the poor are exposed to high levels of lead from chipped lead paints, pollution from automobile fumes, or other excessive exposure to lead in the environment.

3.2. Lower Current Productivity of Adults

Studies have shown that although the cardiovascular and metabolic effects of mild IDA are limited at rest, these effects are quite dramatic during strenuous exercise *(21)*. After treatment with iron, work capacity rapidly returns to normal, and several intervention trials have shown that iron supplementation increased work output among road workers, rubber tappers, tea pickers, and industrial workers *(22)*.

The economies of countries in southeast Asia depend more on industries, such as agriculture, manufacturing, and construction, in which physical labor is a crucial component; iron deficiency can exert a strong negative influence in the productivity of these industries and the country's economy. As urbanization increases, the service and knowledge industries will be increasingly more important for these economies in the next 10 yr, and the need for a more educated labor force will also increase.

In light of this, it is understandable that health and nutrition experts, including those of the World Bank, have attempted to address economists regarding the need for micronutrient interventions in both children and adults to support the economic development in these countries *(23)*.

4. THE INDONESIAN CRISIS

Indonesia is the world's fourth most populous country, with an estimated population of 231 million in 2002. It is a geographically vast country that extends for more than 4800 km from west to east and 2000 km from north to south and is located between the Asian mainland and Australia. It has a highly diverse archipelagic structure consisting of some 17,000 islands, of which 6000 are inhabited. Approximately 55% of Indonesia's total population lives in Java, the most densely populated region. Java is divided into six provinces: Banten, West Java, Central Java, Yogyakarta, East Java, and the capital—Jakarta.

The country is endowed with substantial agricultural potential; thus, agriculture, including forestry and fisheries, is the most important sector of the economy in terms of employment. Additionally, there is a vast range of mineral resources, including oil. Since the mid-1980s, the manufacturing sector has dramatically expanded, and in 1996 its share in gross domestic product (GDP) exceeded that of agriculture.

In the past 30 yr, Indonesia has been frequently hailed as a leading economic success story. For the decade since 1987, real GDP growth averaged more than 7% per year. In 1996,

GDP per capita surpassed $1000, compared with $70 in 1965. The rupiah was stable. Annual inflation was reported in single digits, and foreign capital was pouring in. As a result, the prevalence of poverty, measured against the official poverty line, declined from about 40% in 1976 to 11.3% in 1996—a remarkable decline given that the population increased from 120 million to 195 million. Over the same period, the infant mortality rate declined from an estimated 145 to 52 infant deaths per thousand live births, and the mortality rate for children younger than age 5 yr declined from approx 217 to 75 per 1000. Indonesia was one of the first developing countries to identify that its high levels of severe vitamin A deficiency constituted a serious public health problem. Over the last 30 yr, the country has come a long way in reducing the levels of severe vitamin A deficiency, and prior to the crisis, Indonesia, with the exception of three provinces, no longer considered vitamin A deficiency a public health problem *(24)*.

However, in 1997, Indonesia experienced a severe drought (El Nino), low world oil prices, regional financial instability, domestic social unrest, and, ultimately, a change of government.

The El Nino-induced drought was a remarkable drought because it had two distinct peaks and was extremely severe. It began between February and April 1997 and reached its first peak in July and August. This coincided with large-scale forest fires in Sumatra and Kalimantan. However, only the magnitude (not the origin) of these fires can be blamed on El Nino. Plantation operators, transmigration contractors, timber concessionaries, and, to a smaller extent, small farmers were responsible for land clearing activities using fire. Some 500,000 hectares were affected by the drought, of which 85,000 hectares experienced severe crop losses. In late 1997, the El Nino phenomenon began to develop toward a second peak and continued its increase until March 1998. Total rice production in 1997 fell by 4%, but some provinces in the country registered sharper declines.

By the end of 1996, the macro-economic conditions of Thailand appeared to be very shaky, with large external deficits, increasing short-term foreign indebtedness, and the fragile financial conditions of corporate firms and finance companies that had borrowed heavily abroad to finance the speculative boom in real estate and equity investments. At the end of 1996, the Thai Baht had already come under attack. In the beginning, Indonesia was an unaffected bystander to the crisis in Thailand. However, in August 1997, the Indonesian rupiah began to come under pressure and rapidly depreciated in the following months. The Indonesian rupiah, which was trading 2450 to the US dollar in July 1997, plunged to a low of 17,000 in January 1998 before strengthening to around 8000 in October 1998. Although the United States was in a strong cyclical upswing during the Mexican crisis and was able to help Mexico, the weakness of Japan's economy in 1997 exacerbated the Asian crisis and worsened the unfolding of the currency crisis. For many businesses, exchange rate volatility made planning almost impossible. Annual inflation ran at an estimated 80%. Foreign capital fled, closing off access to new foreign lending. Between 1992 and July 1997, about 85% of the increase in external debt was a result of borrowing in the private sector. This mostly resulted from the continuous economic growth that attracted private foreign resources. With the increasingly unfavorable exchange rates, businesses were struggling to service existing foreign debts.

As soon as it was called upon, the IMF quickly moved to help Indonesia formulate reform programs aimed at tackling the roots of its problems and restoring investor confidence. The Indonesian government signed its first letter of intent to the IMF on

October 31, 1997. However, the economic situation only worsened, and Indonesia's agreement with the IMF was revised several times during the following months. In May 1998, after fuel prices increased and protesting students were shot, riots and looting swept across Jakarta and other cities, leading to the resignation of President Soeharto on May 21 and his replacement by his vice president, B. J. Habibie.

5. HELEN KELLER INTERNATIONAL NUTRITIONAL SURVEILLANCE

The impact of macro-economic adjustment programs and economic crises on food security and the nutritional status of the poor is not well-documented *(25)*. Helen Keller International had extensive experience in establishing natural disaster impact assessment in Bangladesh *(26)*, and in December 1995, Helen Keller International was given the unique opportunity to set up a nutritional surveillance system in the Central Java Province. Data were collected every 3 mo thereafter, for the duration of 1 yr (until January 1997) *(27,28)*. The purpose of that surveillance system was to assess the impact of a large-scale social marketing program that promoted the consumption of vitamin A-rich foods *(28)*. As a response to the crisis, the surveillance system was revived in June 1998, and expanded to seven more provinces: Jakarta, West Java, East Java (including a special focus on the poor in its capital, Surabaya), West Nusa Tenggara (specifically, the island of Lombok), South Sulawesi (including a specific focus on the poor in its capital, Makassar), West Sumatra, and Lampung. The data presented in this chapter were collected between June 1996 and September 2003.

6. CONCEPTUAL FRAMEWORK FOR CAUSES OF MALNUTRITION

In the 1990s, the United Nations Children's Fund (UNICEF) developed a conceptual framework to determine the most likely causes of malnutrition *(29)*. The framework is a very useful concept and consists of the following key elements:

- Nutritional status is identified as an outcome of processes in society.
- Malnutrition is a result of immediate, underlying, and basic causes in a hierarchical manner.
- Delivery services (e.g. feeding programs, oral rehydration therapy, vitamin A supplementation, expanded program of immunization, etc.) primarily address the immediate causes.
- Access to food, adequate care of women and children, and access to basic health services together with a healthy environment, are necessary conditions for nutritional security and are considered the underlying factors. The inadequate access to food may result in shortages of both macro- (carbohydrates, protein, and fat) and micronutrients (vitamins and minerals).
- The basic causes are defined by the economic, political, and ideological superstructure.

We have modified the UNICEF conceptual framework to include factors that affect a country's political and ideological superstructure (*see* Fig. 1) but that may be outside the direct control of the country's government and other national agencies. Here, we will use the conceptual framework to understand how the coping strategies of the Indonesian population work, to examine how food price policies affect malnutrition, and to hypothesize and assess which indicators of nutritional status are the most crisis-sensitive.

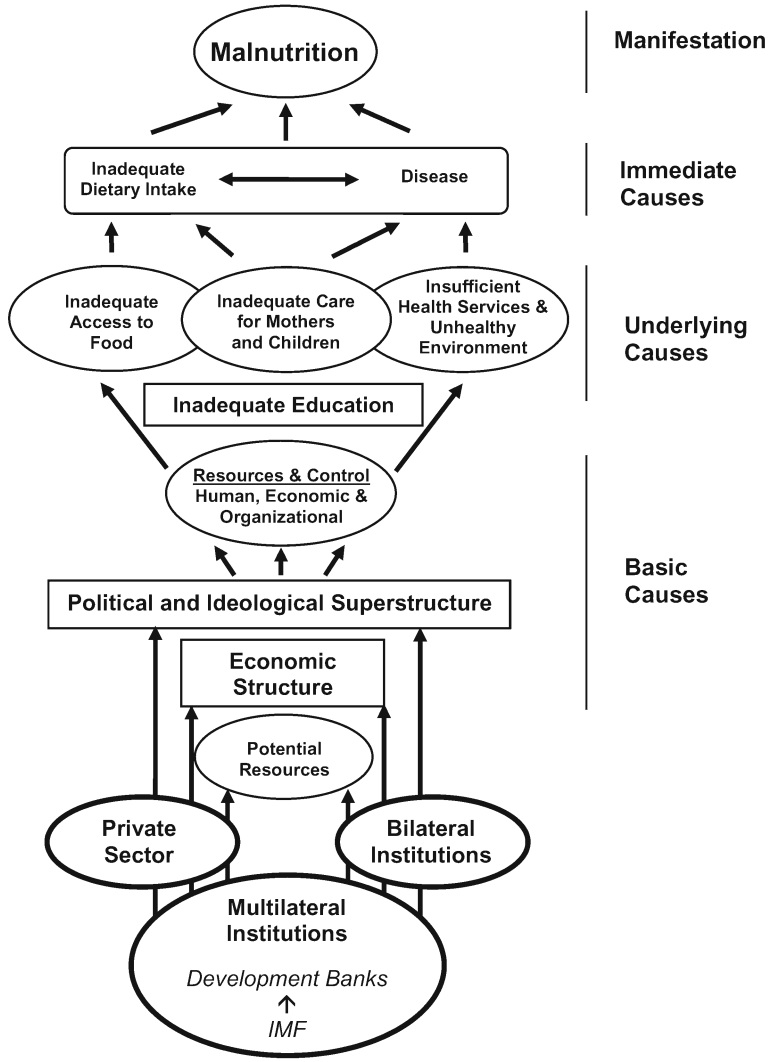

Fig. 1. Conceptual framework of immediate, underlying. and basic causes of malnutrition. (Modified from ref. *29*.)

6.1. Basic Causes

Considering Indonesia's situation since mid-1997, the so-called "basic causes" of the increase in malnutrition were the El Nino drought and the Asian economic crisis. Although the two phenomena are distinct, both had the same effect—namely, a decrease in the real income of many groups of the population. The prices of most food items dramatically increased in the period between January and July 1998, and the inflation rate was 78% in 1998. The economic crisis had serious, adverse social impacts. Urban areas were hit hardest, mainly as a result of massive contraction in the manufacturing and construction sectors in particular. In Jakarta, one of every three workers was unemployed in 1998, and nationwide unemployment reached 20% *(30)*. The high inflation and increased unemployment rates resulted in a sharp decline in real earnings and the

consequent rise in the prevalence of poverty. The proportion of households without any savings increased from approx 60% in June 1998 to nearly 80% in September 1998 *(31)*. Although proportionally the poor were more affected than the middle class, the crisis affected all socioeconomic strata.

A World Bank report also indicated an increase in the prevalence of poverty *(10)*. The World Bank reported that the social impacts were very unequally distributed across sectors and regions. The manufacturing and construction sectors located in the urban and peri-urban areas were hit hardest. Among the regions, Java was most affected.

6.2. Underlying Causes

As mentioned previously, inadequate access to food, inadequate care for mothers and children, and insufficient health services and unhealthy environments are the underlying causes of malnutrition.

What was the impact of increased poverty (reported earlier) on access to food? Although rice prices increased considerably, rice consumption did not change. Also, the poorer households consumed more rice per capita than households of higher socioeconomic status. This implies that the absolute as well as relative expenditure on rice increased.

What happened to the consumption of the more expensive food items, such as animal products? In 1996, the government of Indonesia, in close collaboration with Helen Keller International and UNICEF, carried out a social marketing campaign to promote the consumption of eggs as an important food source for vitamin A. Based on formative research, these groups determined that eggs were the most affordable animal source of micronutrients in Central Java. The program was very successful, indicated by the fact that serum retinol levels, an indicator of vitamin A status, increased as a result of the campaign *(28)*. However, a comparison of the data collected in Central Java before and after the start of the crisis showed that egg consumption decreased as a result of the crisis and that this occurred for all socioeconomic strata *(31)*.

The economic crisis resulted in a decrease in the real income of large segments of the society, and, as a result, these segments were forced to increase their expenditure on food as a percentage of their total expenditure. Although the expenditure on staple foods increased because of unchanged consumption but an increased price, expenditure on more expensive foods, such as animal products, declined.

Data from the Bangladesh Nutritional Surveillance Program of Helen Keller International and the Institute of Public Health and Nutrition showed a very clear relationship between changes of the price of staple food and nutritional status and showed that this was not related to the consumption of the staple food itself but, rather, to the concurrent expenditure on nonstaple foods *(32)*. Figure 2 illustrates how the changes of proportion of underweight children between 1992 and 2000 very closely followed the fluctuation in expenditure on rice. Although rice consumption did not change, a decrease in the price of rice meant that less money was spent to consume the same amount of rice and, therefore, more money was available for the purchase of other food items. As shown in Fig. 3, when non-rice food expenditure increased as a consequence of a lower expenditure on rice, the prevalence of malnourishment decreased. Although malnourishment in children younger than age 5 yr was a very good indicator for change of nutritional status in response to changes of the price of rice in Bangladesh between 1992 and 2000, the issue dealt with whether it would be as sensitive for assessing the impact of the crisis in Indonesia.

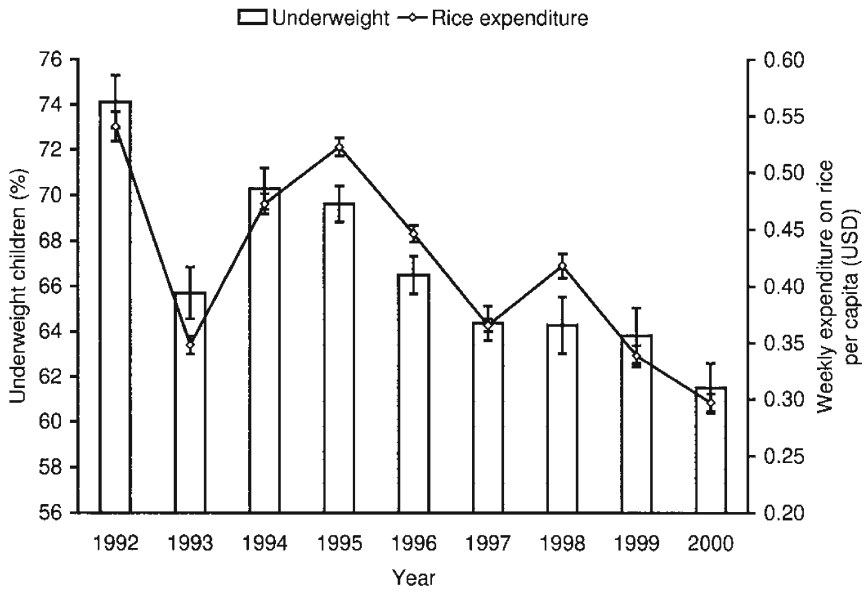

Fig. 2. The percentage of underweight children (Z-score weight-for-age <–2 SDs) ages 6 to 59 mo and the weekly expenditure on rice per capita in US dollars in rural Bangladesh during the month of June 1992 to 2000. Values for underweight are percentage ± 95% confidence interval, and values for expenditure on rice are means ± 95% confidence interval ($r = 0.91$; $p = 0.001$; $n = 9$). (Reprinted with permission from ref *32*. © 2003 American Society for Nutritional Sciences.)

The type of impact that the Indonesian crisis had on the other underlying causes (inadequate care, insufficient health services, and unhealthy environments) is still unclear. Regarding health care services, the vitamin A capsule distribution program, for children unger age 5 yr, is a program that has been in place since the early 1970s. The capsule distribution (as evaluated between June 1998 and January 1999 and compared to 1996) showed only a slight decline in its coverage. Access to health care services that require payment may have declined somewhat more as a result of the reduction of purchasing power and limited availability of safety-net programs, particularly in urban areas. Caring practices may have suffered less, because knowledge is not affected by a crisis, and time available for child care was not likely to have been reduced much because of the increased lack of employment opportunities. Environmental hygiene was also less likely to deteriorate much, because there was no disaster or total disruption of public services. Therefore, it appears that of the underlying causes of malnutrition, the Indonesian crisis mostly affected access to food, primarily resulting in reduced dietary intake.

6.3. Immediate Causes

Of the two immediate causes of malnutrition, dietary intake and morbidity, Indonesia's crisis primarily affected dietary intake through the reduced access to food. Morbidity could have increased as a result of the reduction of quality and quantity of the diet and/or from a possible reduced access to health care services. However, the main impact of the economic crisis was on purchasing power, which largely affected access to food and, therefore, reduced quality and quantity of the diet.

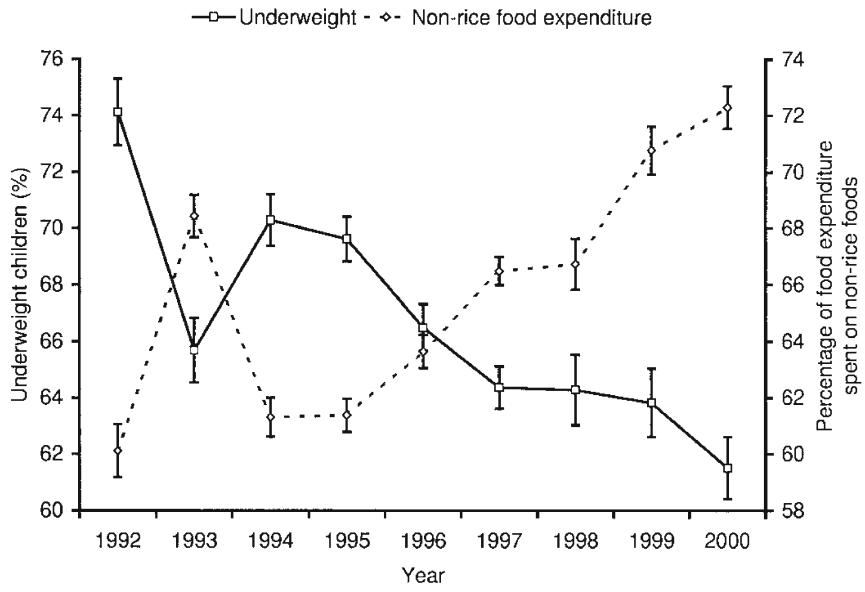

Fig. 3. The percentage of underweight children (Z-score weight-for-age <–2 SDs) ages 6 to 59 mo and the percentage of food expenditure spent on non-rice foods in rural Bangladesh during the month of June 1992 to 2000. Values for underweight are percentage ± 95% confidence interval, and values for expenditure on non-rice foods are means ± 95% confidence interval ($r = 0.91$; $p = 0.001$; $n = 9$). (Reprinted with permission from ref *32*. © 2003 American Society for Nutritional Sciences.)

7. INDICATORS OF CHANGES OF MALNUTRITION RESULTING FROM THE CRISIS

The UNICEF conceptual framework can also be used to hypothesize about the most crisis-sensitive indicators of a change of nutritional status. As explained earlier, the greatest effect of the crisis was a drop in the real income of the population, resulting from the increase in prices of several commodities and from increase in unemployment. Furthermore, the IMF program included measures to reduce government spending on food subsidies and other social programs (*see* bottom part of conceptual framework shown in Fig. 1). The urban population in Indonesia was mainly dependent on the market as the main source of food. Affordable prices of food and the ability to earn a cash income are generally crucial to the achievement of food security in urban areas *(33)*. However, the prices of these food items became two to three times more expensive, and the urban poor hardly had any coping mechanisms for acquiring food in another way. Furthermore, despite the fact that this finding was recognized in September 1998, the safety-net programs of the government mainly focused on the rural areas. Therefore, the impact of the crisis on access to food was larger in the urban than the rural population, and largest among the urban poor. The NSS data of the urban areas were collected from households in the poorest neighborhoods and slums. Most of the people living in these areas had jobs in the construction industry, which was one of the first sectors that collapsed as a result of the economic crisis. The following sections examine the impact of the crisis on the nutritional status of the urban poor of Jakarta.

The diagrams shown in Fig. 4 are derived considerations regarding how reduced purchasing power can affect food intake and how that can affect nutritional status of different

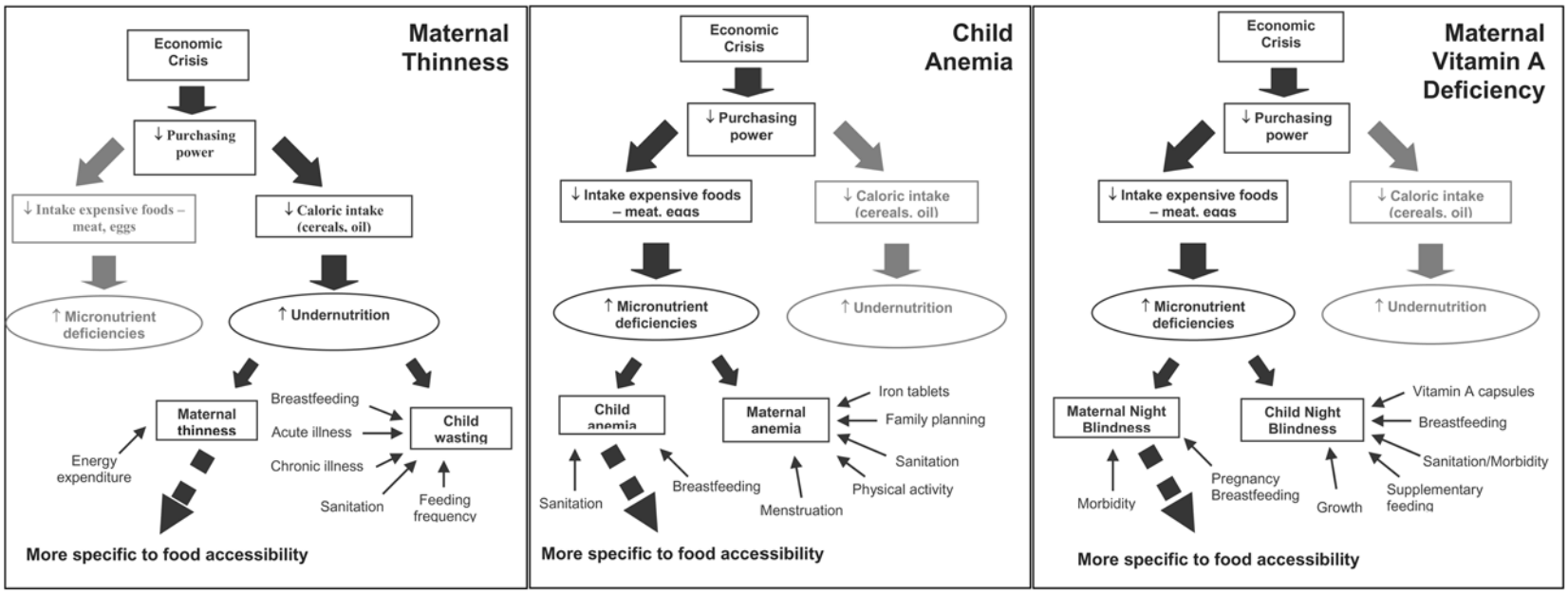

Fig. 4. Relationship between a reduction of purchasing power and indicators of nutritional status: maternal thinness, child anemia, and maternal nightblindness. (Modified with permission from ref.)

members of the population (e.g., women or children) along with what other factors affect their nutritional status *(34)*. When food consumption decreases, both caloric intake (energy) as well as the consumption of more expensive foods, (such as animal foods and industrially produced foods) decrease.

7.1. Maternal Thinness

A reduced caloric (dietary energy) intake can result in a loss of weight among any members of the population. However, mothers' weights likely are affected first, because mothers are more likely to reduce their own food intake before that of their children or husbands. Additionally, as shown in the first diagram of Fig. 4, children's weights are also affected by many other factors, including morbidity and care factors, and may be addressed through crisis-relief programs. Therefore, we hypothesized that an increase of maternal thinness (indicated by a body mass index [BMI] <18.5 kg/m^2) precedes an increase in malnourishment among children and that maternal thinness is a more specific sign of reduced access to food, because mothers' weights are less affected by illness and special relief programs (*see* Fig. 4). Indeed, the surveillance data from Central Java found no difference in the prevalence of malnourishment among children younger than age 5 yr between 1996 and 1998, and similar results were reported by the World Bank, which used the Indonesian Family Life Survey data.

However, findings were different for women. Data from Central Java collected before and after the onset of the crisis (January–December 1996 vs. July–December 1998) showed an increase in maternal thinness from 14.9 to 17.7% and a reduction of 0.46 kg/m^2 in BMI *(35)*. This decrease was almost equivalent to the increase of 0.50 kg/m^2 in BMI that occurred in south and southeast Asia between 1960 and 1990 *(36)*. Data collected among adolescents in East Java showed similar results. Although the BMI of adolescents increased in the year before the crisis (0.27 and 0.22 kg/m^2 among boys and girls, respectively), it remained unchanged in the school year after the start of the crisis *(37)*. Figure 5 shows the changes of the prevalence of maternal thinness as observed among women living in urban slums of Jakarta between November 1998 and September 2003. Approximately 9 mo after the peak of the crisis, the prevalence was at its highest at 16.9%,and since early 2000 has fluctuated between 10 and 14%. This confirms that maternal thinness is a sensitive indicator for reduced access to food as a result of an economic crisis.

Although malnourishment in children was not a sensitive indicator for the consequences of Indonesia's economic crisis, it was very sensitive to the fluctuations of the price of rice in Bangladesh. This discrepancy may result from several reasons. First, prevalence of malnourishment is much higher in Bangladesh than in Indonesia (60–75% vs 15–25%) and, therefore, is more likely to be responsive, because a small change of body weight among children that are underweight (a weight-for-age Z-score below –2) can get them above the cut-off level for malnourishment and thus reduce the prevalence of malnourishment. In Indonesia, a much larger proportion is already well above the cut-off and would need to lose quite a bit of weight before qualifying to be below the cut-off for malnourishment, making it less likely for the prevalence of malnourishment to increase in that population. Second, dietary intake in Bangladesh is much less varied, with even oil intake increasing when rice prices decline; therefore, not only dietary quality (which is not likely to affect body weight) but also dietary quantity (which directly affects body weight) changes more quickly. Finally, children share

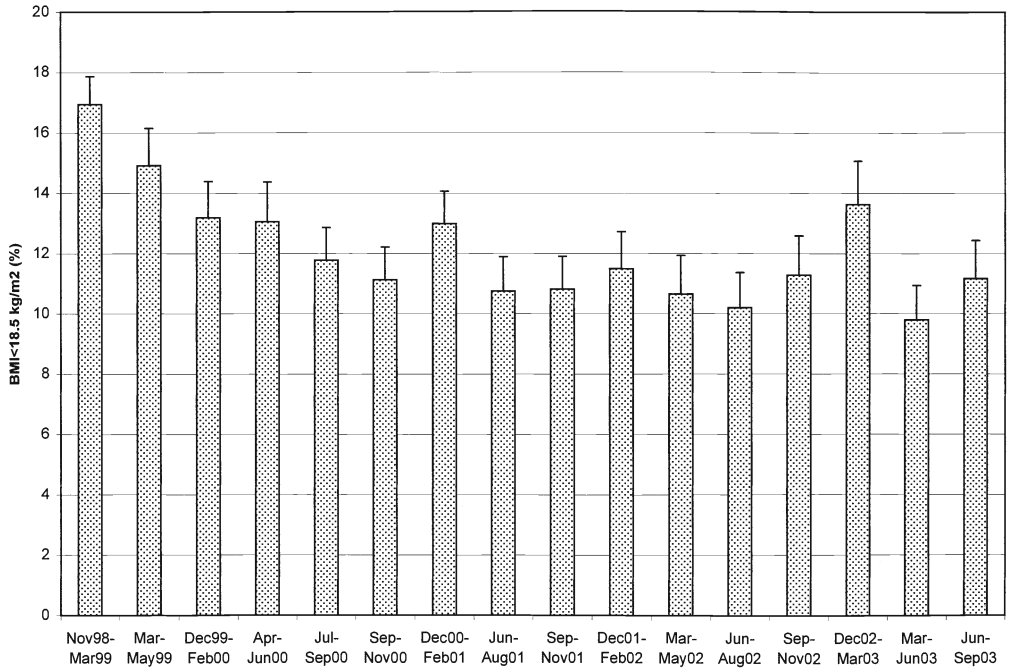

Fig. 5. Prevalence of maternal thinness (BMI <18.5 kg/m^2) among nonpregnant women in urban slums in Jakarta between November 1998 and March 2003 (n = 45,162). Bars indicate 95% confidence intervals, corrected for design effect.

immediately in an improvement of a family's diet but are less likely to be the first to have a lower caloric intake when a family has to reduce the quantity of its diet. Therefore, when rice prices decrease and access to food increases, children's diets also immediately improve. However, in the case of a reduced access to food because of the economic crisis, the quality of children's diets are affected sooner than the quantity, and the quantity of the diet of other family members is affected before that of the children.

7.2. Micronutrient Deficiencies

It is well-established that without fortification or supplementation, communication strategies for dietary behavior change are unlikely to be adequate by themselves in either treating or preventing IDA *(37)*. Meats (beef, pork, lamb, fowl, etc), fish, and liver, which are rich in heme-iron, are the best sources of iron but are also relatively expensive and unaffordable for the poor *(38)*. Similarly, recent research from Indonesia and Vietnam has shown that plant sources of vitamin A are four to five times less effective in improving vitamin A status than previously believed *(39–41)*. Therefore, animal products are essential for improving micronutrient status in poorer populations, especially where the contribution from fortified foods to micronutrient intake is small. Animal products, such as eggs and liver, are rich in micronutrients, including not only iron and vitamin A but also zinc and B-vitamins.

As mentioned previously, the economic crisis showed a decline in the consumption of animal products such as eggs, whereas the consumption of the staple foods remained

the same. Based on these data, an increase of micronutrient deficiencies was expected. We used anemia, which in Indonesia is largely a result of iron deficiency, as a proxy for micronutrient status in general, and we used night blindness, which is the first clinical sign of vitamin A deficiency, as a proxy for more severe forms of micronutrient deficiencies. The second and third diagram boxes in Fig. 4 show that child anemia and maternal night blindness are likely the most sensitive indicators to a change in intake of micronutrient-rich, more expensive foods, because these are not affected much by other underlying factors such as performance of health care services, other feeding or caring practices, varying biological factors, or environmental hygiene.

7.2.1. Iron Deficiency Anemia

Although more than 22 countries have adopted public health policies calling for iron supplementation of infants and preschool children, very few programs exist. Infants born to mothers with IDA are more likely to have low iron stores. In fact, NSS data showed that nearly 40% of infants, ages 3 to 5 mo, in Java are already anemic (Hb <100 g/L) even if they had a normal birth weight and that their risk of being anemic is higher when their mother is anemic *(42)*. Birth weight is another important determinant of an infant's iron status at the time of birth. Therefore, low-birth-weight infants need iron supplementation from age 2 mo up to at least age 24 mo *(43)*. Although breastfeeding remains key to the health and nutrition of infants, after 6 mo, even infants with normal iron stores have used the iron stores they had at birth, and breast milk does not provide the amount needed as they continue to rapidly grow and develop. Thus, the high prevalence of iron deficiency among infants and young children is largely the result of maternal anemia during pregnancy and subsequent inadequate dietary intake to meet the relatively high iron requirements of early childhood. Where children do not widely and regularly consume iron-fortified complementary foods and iron-rich animal foods, IDA in children ages 6 mo to at least 24 mo is virtually certain to be an important public health problem.

There is an array of evidence demonstrating that moderate-to-severe anemia in infancy has a longlasting impact on mental development, whereas iron deficiency at any age has adverse effects on cognitive performance and work capacity *(14–16,44–48)*.

As mentioned earlier, there are no programs in Indonesia that address anemia among young children. Also, as mentioned previously regarding the difference of the prevalence of malnourishment in Bangladesh and in Indonesia, the prevalence of anemia is much larger among young children than among mothers and is therefore more responsive to changes. Because there are also fewer other factors that impact on their likelihood of having anemia as compared to women, child anemia was believed to be a more sensitive indicator of a reduced consumption of micronutrient-rich foods. Figure 6 demonstrates that this was indeed the case; the prevalence of anemia in Central Java before and after the start of the crisis changed more among young children than among mothers. The change of the prevalence of anemia among young children in Jakarta between November 1998 and September 2003 is shown in Fig. 7. At 9 mo after the peak of the crisis, the prevalence of anemia was at its highest and has since declined to about 55 to 65%. However, it appeared to have increased again in 2003, which probably indicates the continuing effects of the Bali Bombing in October 2002 on the stock market and the decrease in the influx of foreign currencies as a result of the decline in tourism.

It should be noted that the prevalence of anemia, even at the observed lower levels, is still extremely high and warrants immediate action. In fact, it is recommended that if

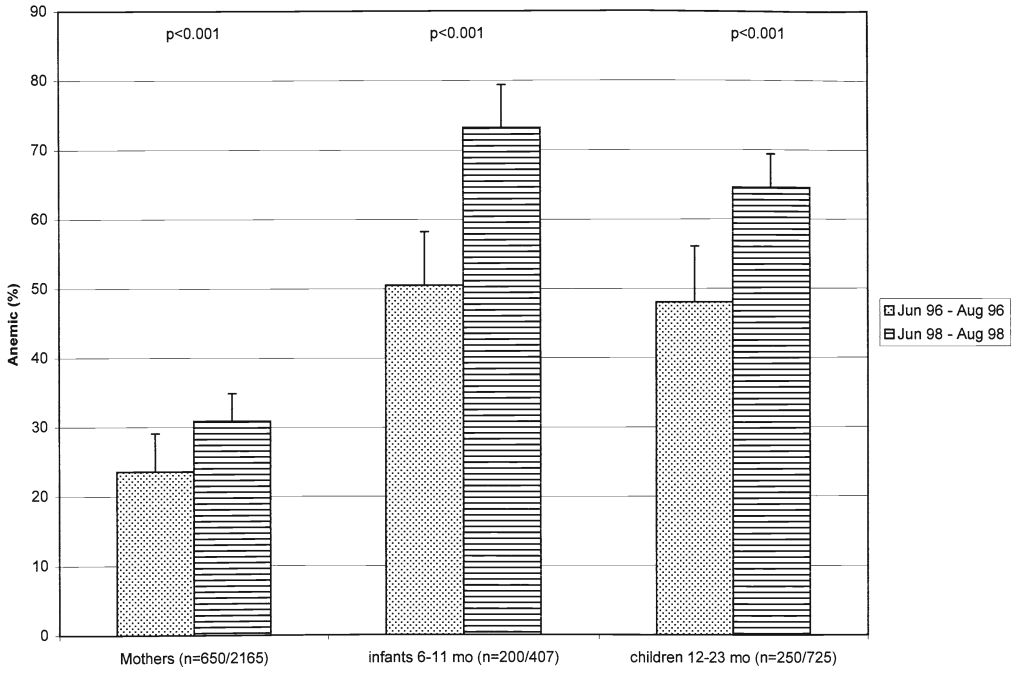

Fig. 6. Proportion of mothers, infants ages 6 to 11 mo, and children ages 12 to 23 mo in Central Java suffering from anemia (using World Health Organization cut-off points for hemoglobin levels) before and after the start of the economic crisis. Bars indicate 95% confidence interval corrected for design effect. $*p < 0.05$, $***p < 0.001$ (Chi-square test).

the prevalence of anemia is greater than 40%, all children should be supplemented daily until age 24 mo *(44)*.

Apart from the devastating impact of iron deficiency and anemia on the infant and young child, these children also are at higher risk of lead poisoning. Similarly to all other megacities of the world, Jakarta has a high level of lead pollution, and the data shown are from children that live in the poorest areas of urban Jakarta, close to the roads. Although we have no data regarding the prevalence of lead poisoning, it can be assumed that the prevalence is very high among these iron-deficient infants and young children.

7.2.2. Vitamin A Deficiency

Vitamin A is required for the proper maintenance and function of the immune system and is essential to ensure the integrity of the respiratory and digestive epithelia that protect people from acute infections. Vitamin A supplementation among children reduces the risk of mortality by 23% *(49)*. A recent study showed that vitamin A supplementation may also reduce maternal mortality by 40 to 50% *(50)*.

In 1978, Indonesia's first national vitamin A survey showed that xerophthalmia was a public health problem, and Indonesia became one of the first developing countries to recognize that its high levels of severe vitamin A deficiency constituted a serious public health problem. The country then began implementing programs to eliminate the

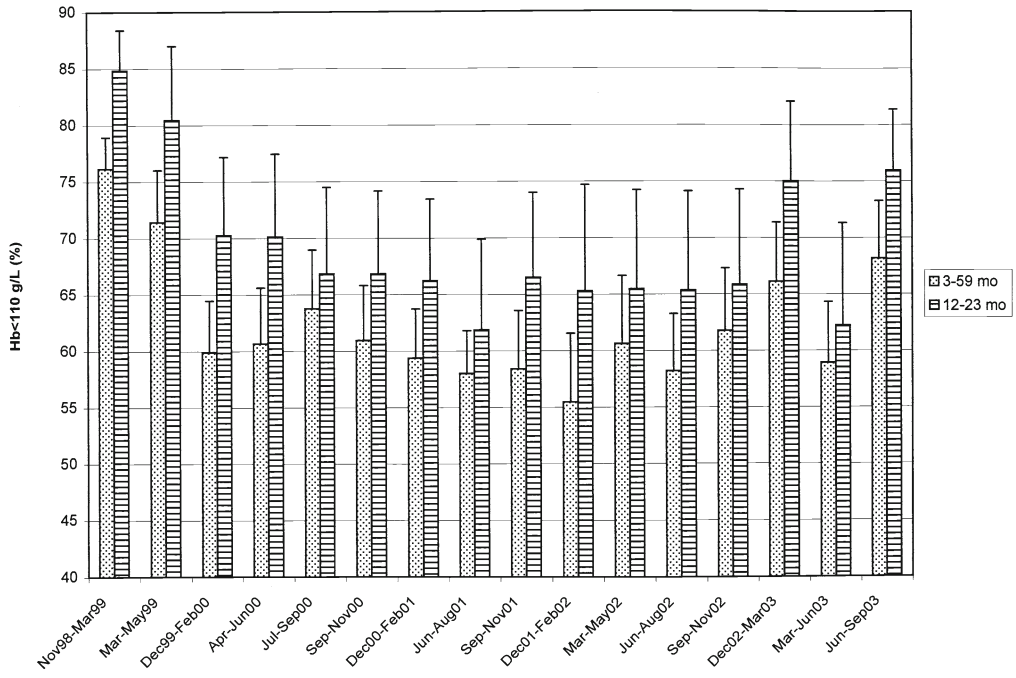

Fig. 7. Prevalence of anemia (Hb < 110 g/L) among children ages 3 to 59 mo and 12 to 23 mo in urban slums in Jakarta between November 1998 and March 2003 (n = 10,707 and 2826, respectively).

problem, including supplementing young children (ages 12–59 mo) through the existing health system. Over the past 30 yr, the country has been considered a major success story in reducing the level of severe vitamin A deficiency, and prior to the crisis, xerophthalmia among children younger than age 5 yr was no longer considered a public health problem, although a considerable part of the population still had serum retinol levels that were too low *(24)*, which was associated with an increased mortality risk.

Although anemia among young children is a very sensitive indicator for a change of dietary quality (as mentioned earlier), their vitamin A status is not because of this successful distribution of high-dose vitamin A capsules twice per year and a relatively unchanged performance of this supplementation program during the crisis. For mothers, however, vitamin A capsule distribution is virtually nonexistent because it is only done within 6 wk after delivery and coverage of that program is low (10–30%, unpublished observations from Helen Keller International). Figure 8 demonstrates that maternal night blindness was at its highest between November 1998 and February 2000. Those levels were comparable to the 2.2% observed among women in rural Bangladesh in 1997 to 1998 *(51)*. Since April 2000, night blindness has declined, reaching 0% in September 2002 and remaining there.

8. INDONESIA: 1999 TO 2003

Indonesia achieved a growth of 0.8% in the GDP in 1999; by 2000, the economic recovery had a broader base, with exports and fixed investment contributing to the turnaround. Similarly to many countries in southeast Asia, however, Indonesia's economic growth experienced a slowdown in 2001. Despite positive news (including the peaceful

Table 1
Major Economic Indicators, Indonesia, 1997–2003

Economic indicator (%)	*1997*	*1998*	*1999*	*2000*	*2001*	*2002*	*2003*
GDP growth	4.7	–13.1	0.8	4.9	3.4	3.7	3.4
Inflation rate	6.2	77.6	–1.9	3.7	11.5	13.1	7.7
Current account balance/GDP	–2.4	4.3	4.1	5.2	3.1	1.5	0.7

(From Asian Development Outlook, 2002: II. Economic Trends and Prospects in Developing Asia. ADB, Indonesia, 2002.

Abbreviations: GDP, gross domestic product.

change in government and the steady, incremental progress in some aspects of economic policy), exports fell, responding quickly to weakening external markets—first, as a result of the bursting technology stock market "bubble," and then as a result of the terrorist attacks in the United States on September 11, 2001.

Signs of some rebound in market confidence were apparent in late 2001 and early 2002, with improvements in stock and bond market indexes. However, although growth accelerated over the first three quarters of 2002, it was set back by the October terrorist attacks in Bali. The currency and stock market reacted sharply, and the effects of the consequent decline in tourism were observed in early 2003. This downturn, monitored by financial observers such as the Asian Development Bank, coincided with an increase of childhood anemia and maternal thinness observed by the Helen Keller International surveillance system (*see* above).

The bombing of the J.W. Marriot hotel in Jakarta in September 2003 may have further contributed to similar uncertainties in the economic and social situation, and the elections scheduled for mid-2004 may involve an increasingly politicized debate on economic policy. It is clear is that the country remains vulnerable to external shocks, and it is essential to monitor the impact of such shocks on the health and nutritional status of vulnerable groups in Indonesia.

9. CONCLUSIONS AND RECOMMENDATIONS

The first health impact of the Indonesian economic crisis was observed on food intake, as the consumption of animal products decreased to maintain the consumption of the main staple, rice. This resulted in a dramatic increase in the prevalence of childhood anemia in both urban and rural parts of Indonesia. Because animal products are rich in other micronutrients as well, the data suggest that affected children are also likely to be deficient in these other micronutrients. IDA among children in areas without extensive iron fortification efforts and/or iron supplementation programs proved to be a very sensitive indicator for measuring the change and impact of structural adjustment programs or of economic crises similar to that in Indonesia (Fig. 7).

The urban areas were more affected by the Indonesian crisis than the rural areas. The Indonesian economic crisis resulted in the collapse of the construction sector and the increase of food prices. Because the urban poor were dependent on food prices and the ability to earn cash income, we predicted an increase in malnutrition rates. About 30% of Jakarta's urban poor are not officially registered as citizens of Indonesia's capital city, perhaps explaining why safety-net programs did not prevent the most vulnerable urban groups from falling into poverty.

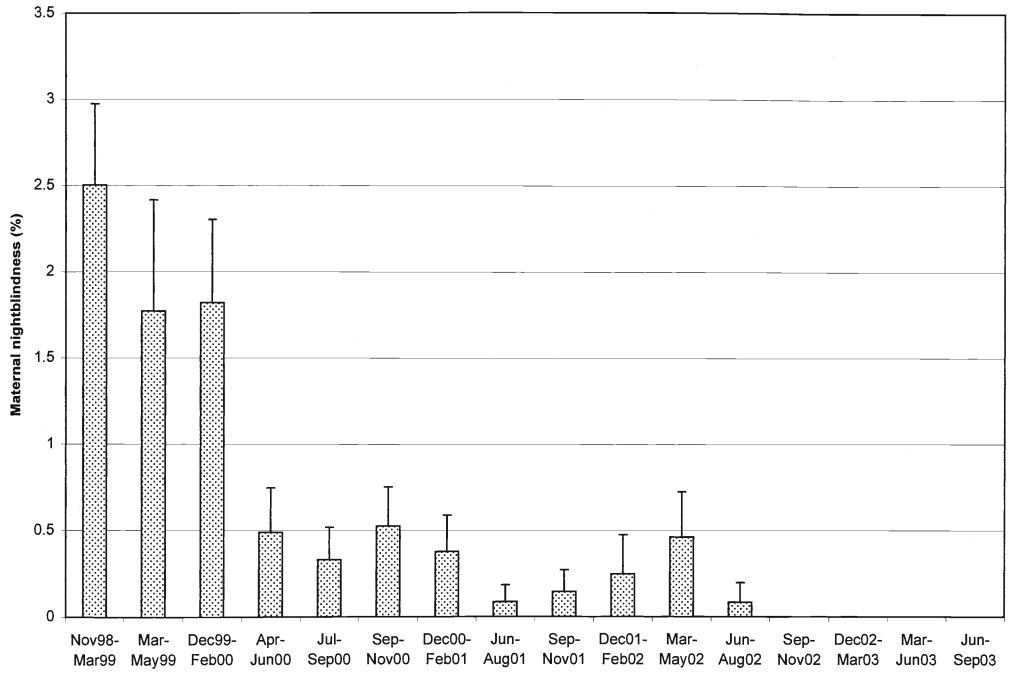

Fig. 8. Prevalence of nightblindness among non-pregnant women in urban slums in Jakarta between November 98 and March 2003 (n = 45,148).

During the crisis, however, the vitamin A capsule distribution program remained mostly intact, which is presumed to account for the fact that there was only a slight increase detected in childhood night blindness (data not shown). Mothers generally do not have access to vitamin A capsules, which is why a change in the prevalence of maternal night blindness has proved to be a more useful indicator in such situations but is still less sensitive for monitoring change than maternal thinness and/or childhood anemia (Fig. 8).

9.1. Recommendations

According to Stiglitz, an economy in deep recession may grow faster as it recovers, but it never makes up for the lost time. There seems to be an association between the depth of the recession and future income. Therefore, the effects of a recession are longlasting *(9)*. Similarly, the data on childhood anemia showed that the impact of an economic crisis on the immediate health status of children will also have longlasting effects on the human capital of countries.

The Indonesian crisis had specific effects on the nutritional and health status of its population. There are many lessons to be learned from these data. Based on what we have learned so far, it is recommended that in such economic crises, the most efficient way of detecting nutritional distress—and hence, likely increased morbidity and impaired development—is to track the following indicators: child hemoglobin levels, maternal night blindness, and maternal thinness. It is less useful to rely on signs of protein-energy malnutrition, as has been done customarily in other forms of emergencies.

In 1993, the World Bank published its report, Investing in Health. The report highlighted the extent to which health interventions were cost-effective, at least in economic

terms. Besides the fact that micronutrient deficiencies may result in an increased risk of both childhood and maternal mortality, these deficiencies also have a longer term impact on poor development of human resources (i.e., of people not being allowed to reach their intellectual and growth potential). This is something that is very often forgotten in economic adjustment programs, and we would argue for more emphasis on micronutrient deficiency control as part of safety-net programs.

Countries cannot afford to lose a generation. Iron deficiency has a massive but, until recently, almost totally unrecognized economic cost *(14)*. In 2004, Indonesia only partly recovered from the economic crisis; however, it already must compete economically both with other countries in the region and globally (most specifically with the People's Republic of China and Vietnam) *(52)*. This competition depends, to a large extent, on human resources and the adequacy of their health and nutrition.

Williamson noted that the Washington Consensus was forged in a time when economists were convinced that the key to economic development was in economic policies rather than in physical and human capital. This view of development is increasingly being challenged by critics, such as Stiglitz, who have observed that economic policy prescriptions imposed on Indonesia by the IMF and intended to lead the country out of its crisis led, in fact, to more instability. As the Helen Keller International surveillance system found, this instability contributed to large-scale micronutrient malnutrition, which will have longlasting effects on the human capital of the country and will diminish its potential recovery. The lessons from the Indonesian crisis are of critical importance for many other Third World countries, which will be struggling in the next 15 yr to reach their millennium development goals.

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VIII Preventive Nutrition: Global Perspectives

28 Potential Benefits of Preventive Nutrition Strategies

Lessons for the United States

Walter C. Willett

KEY POINTS

- Staying lean and physically active throughout adult life has major health benefits.
- Diets low in the percentage of energy from fat have not been associated with lower risks of heart disease, cancer, or better long-term weight control.
- Replacing *trans* and saturated fats with unsaturated fats substantially reduces risks of heart disease.
- Consuming grains in their original high-fiber/whole grain form is likely to reduce risks of type 2 diabetes and heart disease.
- High intake of fruits and vegetables reduces risks of cardiovascular disease, but the benefits for cancer reduction appear modest.

1. INTRODUCTION

For years, the Recommended Daily Allowances (RDAs) served as nutritional guidelines for individuals and institutions *(1)*. However, the RDAs developed from minimalist criteria primarily aimed at prevention of clinical deficiencies. These guidelines were later supplemented with recommendations to reduce dietary fat and cholesterol. In recent years, attention has focused on specific types of fat and suboptimal intake of dietary factors, even in the absence of recognized clinical deficiency. Dietary factors, including substances not generally considered to be nutrients, now appear involved in the cause or prevention of conditions as diverse as coronary heart disease (CHD), stroke, many cancers, cataracts, and birth defects. The National Research Council (NRC), the Surgeon General's Office, and the US Dietary Guidelines Committee *(2–4)* examined the relation of diet to health more broadly and issued new recommendations. This brief overview builds on the 1989 NRC Report on Diet and Health *(2)* and emphasizes subsequent findings.

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2. SOURCES OF EVIDENCE

Traditionally, animal experiments and small human metabolic studies formed the basis of dietary recommendations. Inevitably, the study of chronic disease in humans has required epidemiological approaches. Initially, investigations compared dietary intakes and disease rates among populations in various countries. These analyses highlighted the large differences in disease rates worldwide and provided many hypotheses; however, such studies were limited, because many other factors, besides diet, varied across cultures, and the data were inherently aggregated. The next generation of studies were primarily case—control investigations, which mainly examined dietary factors retrospectively regarding risk of cancer and other diseases. Now, large prospective studies involving many thousands of people are beginning to provide data based on both biochemical indicators of diet and dietary questionnaires that have been rigorously validated *(5)*. Prospective studies are less subject to biases that result from the retrospective reporting of dietary intakes or the effects of disease on biochemical indicators. Micronutrient supplements can potentially be evaluated in randomized trials; however, trials of dietary interventions often may be infeasible because of difficulties in maintaining compliance for long periods, which are necessary and could last decades. Recent advances in molecular biology have yet to contribute substantially to dietary recommendations; however, in the future, these approaches may provide useful intermediary endpoints, allow the study of gene—diet interactions, and enhance our understanding of the mechanisms by which dietary factors influence disease. Ultimately, our knowledge is best based on a synthesis of epidemiological, metabolic, animal, and mechanistic studies.

3. SPECIFIC DIETARY COMPONENTS

3.1. Dietary Fat

Until recently, major reviews on diet and health have consistently recommended reducing total fat intake, usually to 30% of energy or less *(2,3)*, to decrease CHD. The classical diet—heart hypothesis has rested heavily on the repeated observation that serum total cholesterol levels predict CHD risk; thus, serum cholesterol has functioned as a surrogate marker of risk in hundreds of metabolic studies. These studies, summarized as equations by Keys *(6)* and Hegsted *(7)*, indicated that compared to carbohydrates, saturated fats and dietary cholesterol increase and polyunsaturated fat decreases serum cholesterol, whereas monounsaturated fat has no influence. These widely used equations, although valid for total cholesterol, have become less relevant as surrogate variables for CHD risk with the recognition that the high-density lipoprotein (HDL) cholesterol fraction is strongly and inversely related to CHD risk and that the ratio of total cholesterol to HDL is a better predictor *(8–11)*.

Substitution of carbohydrate for saturated fat (the basis of the American Heart Association [AHA] diets) tends to reduce HDL as well as total and low-density lipoprotein (LDL) cholesterol; therefore, the ratio does not change appreciably *(12)*. In contrast, substituting monounsaturated for saturated fat reduces LDL without affecting HDL, thus providing an improved ratio *(12)*. Additionally, compared to carbohydrates, monounsaturated fats reduce blood sugar and triglycerides in adult-onset diabetics *(13)*. Questions have been raised regarding whether the reductions in HDL caused by a high-carbohydrate diet have the same adverse effect as reductions caused by other factors *(14)*. Although this issue is

difficult to address directly, other factors that influence HDL levels—including alcohol, estrogens, obesity, smoking, exercise, and medications—affect CHD risk in the predicted direction *(15,16)*. The use of the usual cholesterol prediction equations has been further complicated by the recognition that different saturated fats vary in their influence on LDL levels: 18:0, stearic acid (the main fat in chocolate and a major saturated fat in beef fat) has little effect; 16:0, palmitic acid (the main fat in palm oil also found in beef fat) modestly increases LDL; and 14:0, myristic acid (the main saturated fat in butter and other dairy fats) most strongly increases LDL *(11,17)*. However, this usually does not have practical importance because intakes of the various saturated fats are strongly correlated with each other, and there is no good evidence that stearic acid is less atherogenic *(18)*.

The optimal amount of polyunsaturated fat intake in the diet remains uncertain. The earlier metabolic studies predicting total serum cholesterol *(6,7)* suggested that intakes should be maximized, and the AHA recommended intakes of 10% of energy (compared to US averages of about 3% in the 1950s and 6% presently). Concerns have risen from animal studies in which ω-6 polyunsaturated fat (typically as corn oil) has promoted tumor growth *(19)* and from the possibility that high intakes of ω-6 relative to ω-3 fatty acids might promote coronary thrombosis *(20,21)*. However, as described here, available evidence from human studies has not supported these concerns at levels of ω-6 fatty acid intake up to about 10% of calories. Many studies have examined dietary fat in relation to CHD incidence. In Keys' pioneering study of diets and CHD in seven countries *(22,23)*, total fat intake showed little association with population rates of CHD; indeed, the lowest rate occurred in Crete, which had the highest fat intake as a result of the large consumption of olive oil. However, saturated fat intake was positively related to CHD in Keys' study. In contrast to international comparisons, little relationship has been demonstrated with saturated fat intake in prospective studies of individuals *(5,24,25)*. However, some studies tend to support a modest association between dietary cholesterol and CHD risk *(26)*, and inverse associations have been found with polyunsaturated fat *(24,25)*. Similarly, dietary intervention trials generally have shown little effect on CHD incidence when carbohydrate replaces saturated fat, but replacing saturated fat with polyunsaturated fat has reduced the incidence of CHD *(27–30)*. In the trial that most clearly reduced CHD mortality *(31)* in an initially healthy population, many components of the diet, as well as weight and smoking, changed simultaneously.

High intake of ω-3 fatty acids (found primarily in marine fish but also in some vegetable oils and plants) reduces platelet aggregability and prolongs bleeding time *(21)*, slightly reduces blood pressure *(32)*, decreases serum triglycerides, and increases LDL-cholesterol *(33)*. Consumption of fish was associated with a greatly reduced risk of myocardial infarction (MI) in one prospective study *(34)* and in a randomized trial among postinfarction patients *(35)*. Subsequent data have been less supportive of a major effect of fish consumption on overall risk of CHD *(36–38)*, but the benefits of ω-3 fatty acids appear to occur primarily in prevention of fatal arrhythmias that can complicate CHD, rather than in prevention of infarction *(39–41)*. The amount of ω-3 fatty acids needed to prevent arrhythmia is remarkably small—on the order of 1 g per day *(41)*.

Trans-fatty acids are formed by the partial hydrogenation of liquid vegetable oils during the production of margarine and vegetable shortening and can account for as much as 40% of these products. Intake of partially hydrogenated vegetable fats (which increased from nothing in 1900 to a peak of about 5.5% of total fat by about the 1960s) has closely paralleled the epidemic of CHD during this century; this has occurred in

contrast to intake of animal fat, which has steadily declined over this period *(42)*. *Trans*-fatty acids increase LDL, decrease HDL *(42–47)*, and raise lipoprotein(a), another lipid fraction implicated in the etiology of CHD *(46,48)*. Positive associations between intake of *trans*-fatty acids and CHD were demonstrated among regions in the Seven Countries Study *(49)*, a case—control study of men and women *(50)*, and a cross-sectional angiographical study *(51)*. In a multicenter European case—control study, adipose levels of *trans*-fatty acids were, by far, the lowest in Spain, which is also the country with the lowest CHD rates *(52)*. In the same study, the risk of CHD within countries was 40 to 50% higher among individuals with the highest *trans*-fatty acid levels, but this did not quite obtain statistical significance. In the most detailed prospective study, *trans*-fatty acid intake was strongly associated with risk of CHD *(25)*, and, as predicted by metabolic studies, this association was stronger than for saturated fat. The association between *trans*-fatty acid intake *(53)* and risk of CHD has been confirmed in other prospective studies, and the Food and Drug Administration has announced that by 2005, food labels will be required to include the *trans*-fat content (*see* Chapter11).

Understanding of the interrelationships among dietary fats, blood lipids, and CHD risk has been further complicated by the recognition that antioxidants may play a critical role in preventing atherosclerosis. Experimental evidence suggests that lipid-soluble antioxidants such as vitamin E can block the oxidative modification of LDL, an important step in atherogenesis *(54)*. Within Europe, countries with higher blood antioxidant levels have lower rates of CHD *(55)*, and, as described here, vitamin E supplements have been inversely associated with CHD risk, although not in several large randomized trials. Because liquid vegetable oils, particularly those that are minimally processed, are the primary source of vitamin E in our diets, reduction of these fats could have adverse effects on CHD risk. Soybean oil may be an exception, because although it is highly polyunsaturated, its primary form of vitamin E, γ-tocopherol, is rapidly excreted and poorly incorporated into tissues and lipoproteins. Therefore, diets high in soybean oil, the major fat in the US diet, might result in LDL that is particularly susceptible to oxidation. In contrast, LDL particles formed from a diet high in monounsaturated fat in the form of olive oil appear to be relatively resistant to oxidation *(56,57)*.

3.2. Dietary Fat and Cancer

Anticipated reductions in the risk of cancers of the breast, colon and rectum, and prostate provide another major justification for decreasing dietary fat *(2,58)*. The primary evidence for this reduction has been that countries with low fat intake (also the less affluent areas) have had low rates of these cancers *(58,59)*. These correlations have been demonstrated primarily with animal fat and meat intake, rather than with vegetable fat consumption.

The hypothesis that fat intake increases breast cancer risk has been supported by most animal models *(60,61)*, although no association was shown in a large study that did not use an inducing agent *(62)*. Moreover, much of the effect of dietary fat in the animal studies appears to be to the result of an increase in total energy intake, and energy restriction profoundly decreases incidence *(19,60,62)*. In most case—control studies, no association between fat intake and breast cancer was observed, although a weak positive association (relative risk approx 1.07 for 40 vs 30% of energy from fat *[63]*) was found in the pooled data from 12 such studies *(64)*. However, the relevance of these studies is now diminishing because data from many large prospective studies have been published, including approx 8000 cases involving more than 300,000 women

(65–71). None of these studies showed significant elevation of the risk of breast cancer among those with the highest fat intake, and the summary relative risk for the highest vs lowest category of dietary fat composition was 1.03 *(71,72)*. In the largest study *(73)*, no reduction in risk was seen even below 20% of energy from fat. Therefore, over the range of fat intake consumed by middle-aged women in these studies (which included the present dietary recommendations), dietary total fat did not appear to increase breast cancer risk. Recently, higher intake of animal fat, but not vegetable fat, was associated with a greater risk of breast cancer, suggesting that some components of animal food rather than fat *per se* may increase risk *(74)*.

The large (approximately fivefold) differences in breast cancer rates among countries probably result from many factors. High consumption of soy products, which contain anti-estrogenic isoflavonoids *(75)*, has been hypothesized to account for the low rates of breast cancer in Japan. Although deserving further research, similarly low rates of breast cancer are uniformly seen in nonindustrialized countries, most of which do not consume soy products. No relationship between intake of soy products and breast cancer risk was seen in two earlier case—control studies in China *(76)*, but a more recent study suggested that soy consumption during adolescence may reduce risk *(77)*. Indirect evidence is growing to support the finding that the powerful effect of energy and growth restriction on mammary cancer incidence in animals also applies to humans. Adult height (in part a marker of early energy balance) is associated with breast cancer rates both internationally and in many case—control and cohort studies *(78–80)*. Part of this effect is mediated through delayed ovulation *(81)*; childhood weight gain is the primary determinant of age at which menstruation occurs *(82)*, which still averages about 18 yr in China *(83)*.

Associations between animal fat consumption and colon cancer incidence have been documented in some *(84–86)*—but not all *(87)*—studies, whereas little relation has been demonstrated with vegetable fat. Positive associations with animal fat also have been observed for adenomatous polyps, a precursor lesion *(88)*. However, the associations between red meat consumption and colon cancer have been even stronger than the association of fat in some analyses *(85,86)*, suggesting that relationships with red meat may be caused by other components of cooked flesh, such as heat-induced carcinogens *(89)* or the high content of readily available iron *(90)*. Associations between processed meat and colon cancer have been stronger than with total red meat *(91)*.

Similarly to breast and colon cancer, prostate cancer rates are much higher in affluent compared to poor and Eastern countries *(59)*. More detailed epidemiological studies are few; in subsets of several case—control studies, men with prostate cancer reported higher fat intake than did controls *(92,93)*, and associations with red meat consumption have been suggested in some prospective studies *(94,95)*. A positive association has been observed between intake of α-linolenic acid risk of prostate cancer; this association is primarily attributable to consumption of fat from red meat *(96)*.

3.3. Dietary Fat and Body Fatness

Excessive weight is an important cause of morbidity and mortality (*see* Section 4.), and short-term studies have suggested that reducing the fat content of the diet induces weight loss. However, population differences in weight do not appear to be result primarily from fat intake; in Europe, southern countries with relatively low fat intakes have higher rates of obesity than northern European countries *(97)*. Also, among 65 counties in China, no correlation was observed between body weight and fat intake,

which varied from approx 6 to 30% of energy *(83)*. Inconsistent associations have been observed in cross-sectional and prospective studies within countries, but such observations are particularly prone to distortion because subjects may alter their diets to modify their weight. In randomized trials of fat reduction (the optimal way to study this relationship), modest weight reductions typically are seen in the short-term. However, in randomized studies lasting 1 yr or longer, reductions in fat to the range of 20 to 25% of energy had minimal effects on overall long-term body weight *(98–101)*. In a trial among obese women, a reduction in fat intake to less than 18% of energy reduced weight by only 3.4 kg, and no effect was observed on waist/hip ratio or percent body fat *(102)*. Very low fat intakes (less than 10% of energy), in conjunction with a high volume of bulky food (as consumed by some traditional societies), may induce weight loss *(103)*, but long-term studies are needed to confirm this finding. However, available evidence suggests that reductions in dietary fat composition over the ranges currently recommended are not likely to have sustained and substantial effects on body fatness.

What can we currently say about dietary fat and health? As noted—but generally ignored—in the executive summary of the NRC report *(98)*, there is little evidence that dietary fat *per se* is associated with risk of CHD. Metabolic and epidemiological data are consistent in suggesting that intake of partially hydrogenated vegetable fats should be minimized. Metabolic and epidemiological data support a reduction in saturated fats, particularly from dairy sources, to as low as feasible; however, these data suggest that the benefits will be small if carbohydrate, rather than unsaturated fats, replace the saturated fat. Definitive data are not available regarding the optimal intake of polyunsaturated and monounsaturated fats, but the metabolic data as well as the experience of southern European populations suggest that consuming a substantial proportion of energy as monounsaturated fat would be desirable. Available evidence also suggests that total fat reduction would have little effect on breast cancer risk, although reducing red meat intake may well decrease the incidence of colon cancer.

3.4. Vegetables and Fruits

Recommendations to eat a generous amount of vegetables and fruits *(2)* are supported by epidemiological data regarding both cardiovascular and cancer incidence. In more than 200 studies, persons consuming higher amounts of these foods or displaying higher levels of carotenoids in their blood experienced reduced risk of various malignancies *(104,105)*. However, most of these studies were case–control investigations, and more recent cohort studies have tended to show much weaker—or no—relationships between overall fruit and vegetable consumption and risks of common cancers *(106)*. An inverse relationship with lung cancer had been most strongly suggested *(107)*. This led to the suggestion that β-carotene might be the protective factor *(108)*, but this was not supported by randomized trials *(109–111)*. Intake of fruits and vegetables also has been related to lower risk of stomach cancer in many case—control studies *(3,105,112)*, although not in a prospective study *(113)*; mechanistic studies *(114)* suggest a possible protective role for vitamin C. Vegetable and fruit consumption has also been inversely related to risk of colon cancer *(115)* in case–control studies, but, again, this was not supported in a large prospective analysis *(106)*. Considerable evidence suggests that folic acid reduces risk *(86,116)*, but vitamin supplements and fortification are now greater sources than fruits and vegetables in the United States. Although there are fewer studies to support apparent protective effects of greater fruit and vegetable

consumption, these effects also have been observed for cancers of the oral cavity, larynx, pancreas, bladder, and cervix *(104,105,112)* but require prospective confirmation. In prospective studies, there appears to be little relationship between consumption of fruits and vegetables and risk of breast cancer *(117)*.

In addition to the micronutrients noted earlier, plants contain numerous components that have potential anticancer activity *(105)*; such chemicals could reduce the formation of carcinogens, induce detoxifying enzymes, and block the effects of endogenous estrogens. Further details about the amounts of these substances in foods could permit more informative investigations, as lumping fruits and vegetables together has little biological rationale.

In the growing literature on CHD, fiber intake consistently has been related to decreased risk *(5,118–120)*, but the possibility that this association could have resulted from other factors in plants generally has not been explored. Evidence that elevated blood homocysteine is an independent risk factor for CHD and cerebrovascular disease *(121–123)* and that levels can be reduced by supplements of folic acid and vitamin B_6 *(124,125)* suggest one mechanism. Vegetarians generally have reduced blood pressures, and higher intake of fruits and vegetables has been associated with lower blood pressure even in nonvegetarians *(126)*; the active factor remains unclear, but potassium is a likely contributing factor *(127)*.

Suboptimal dietary folic acid, which is mainly obtained from fortified breakfast cereals, vegetables, and fruits, definitively increases risk of neural tube defects (NTDs), the most common severe birth defect *(128,129),* and may account for more than half of these cases. The effect of low folate intake may be particularly adverse among the approx 10% of the population that is genetically less efficient in using the ingested form of this vitamin *(130)*.

In both case–control *(131)* and prospective studies *(132,133)*, intake of dietary antioxidants, including the carotenoids lutein and zeaxanthin, and vitamin C has been inversely related to risk of cataracts. Because cataract formation, which is increased by sunlight and cigarette smoking *(134)*, involves the accumulation of oxidized and denatured proteins, this lesion may represent a convenient marker of long-term oxidative damage. High intake of lutein and zeaxanthin in the form of spinach has been associated with a decreased risk of advanced macular degeneration *(135)*. This is particularly notable, because lutein and zeaxanthin are the carotenoids specifically concentrated in the macula, where they apparently play a protective role against photodamage *(136)*.

3.5. Starches and Complex Carbohydrates

Because protein varies only modestly across a wide range of human diets, a higher carbohydrate consumption is, in practice, the reciprocal of a low-fat diet. For reasons discussed under the topic of fat (*see* Section 3.1.), a high-carbohydrate diet may have adverse metabolic consequences. In particular, such diets are associated with an increase in triglycerides and a reduction in HDL cholesterol *(11)*. These adverse responses may be aggravated in the context of insulin resistance *(137,138)*, which is highly prevalent in Western populations.

Although direct evidence for benefit in reducing disease risk is limited, several reasons exist to emphasize whole grain and other less-refined complex carbohydrates as opposed to the highly refined products and sugar that are generally consumed in the United States. Adverse consequences of highly refined grains appear to result both from the rapid digestion

and absorption of these foods as well as from the loss of fiber and micronutrients in the milling process. The glycemic response after carbohydrate intake, which has been characterized by the glycemic index, is greater with highly refined foods as compared to less-refined, whole grains *(139)*. The greater glycemic response resulting from highly refined carbohydrates is accompanied by increased plasma insulin levels and augment the other adverse metabolic changes caused by carbohydrate consumption *(139)* to a greater degree than with less refined foods. Diets with a high glycemic index, particularly when associated with low-fiber intake, appear to increase the risk of noninsulin-dependent diabetes *(140,141)* and, possibly, the risk of CHD, particularly among women with greater insulin resistance *(142)*. Anticipated reductions in colon cancer risk from diets high in grain fiber have been difficult to document epidemiologically *(143–145)*. However, reduced constipation and risk of colonic diverticular disease *(134)* are clear benefits of such diets. The role of soluble fiber (found in oat bran and some other plant foods) in lowering blood lipids has been the topic of hot debate; current evidence suggests that a small effect may exist with large intakes *(146,147)*. Risk of MI appears to be reduced by higher intake of dietary fiber from grains to a greater degree than can be explained by the effect of fiber on blood lipids alone *(148)*.

The importance of micronutrients in the prevention of many chronic conditions (discussed in Section 6.) has re-emphasized the problem of "empty calories" that are associated with diets high in sugar and highly refined carbohydrates. In the standard milling of white flour, as much as 60 to 90% of vitamins B_6 and E, folate, and other nutrients are lost *(149)*; this may be nutritionally critical for persons with otherwise marginal intakes. Thiamin, riboflavin, folate, and niacin presently are replaced by fortification, but other nutrients remain substantially reduced.

3.6. Protein

Average protein consumption in the United States substantially exceeds conventional requirements *(2)*, and adequate intake can be maintained on most reasonable diets, including those without animal products. High intake of animal protein can increase urinary calcium loss *(150)*, contribute to homocysteinemia *(151)*, and has been hypothesized to increase risk of various cancers *(152)*; however, evidence for the latter effect is limited. Substituting protein for carbohydrate improves blood lipids and has been associated with lower risk of CHD *(153)*.

3.7. Calcium and Dairy Products

Recommendations to maintain adequate calcium intake *(2)* and to consume dairy products on a daily basis *(154)* are derived primarily from the importance of calcium in maintaining bone strength. Calcium supplements, in conjunction with vitamin D, have reduced fracture incidence in older adults *(155,156)*, but benefits of calcium cannot be distinguished from those of vitamin D. Uncertainty remains regarding the optimal intake. Intakes as high as 1500 mg/d have been recommended for postmenopausal women who are at risk of fractures *(157)*; these intakes are difficult to achieve without supplements. However, many populations have low fracture rates despite minimal dairy product consumption and low overall calcium intake by adults *(158)*. Milk and other dairy products may not be directly equivalent to calcium from supplements, because these foods contain a substantial amount of protein, which can enhance renal calcium losses *(150)*. Several prospective studies have directly addressed the relationship of

dairy product consumption to fracture incidence; with the exception of one small study *(159)*, higher consumption of calcium or dairy products as an adult has not been associated with lower fracture incidence *(160,161)*. At best, the benefits of high calcium intake are minor compared with those from regular physical activity *(162–165)*.

Inverse associations have been reported between calcium intake and blood pressure in some studies *(166)*, but in a review of trials of supplementation, little overall effect was observed *(167)*. Low calcium intake has been associated with risk of colon cancer, although evidence has not been consistent *(168)*. Larger prospective studies suggest a modest benefit of higher calcium, but the relationship appears nonlinear, with most benefit achieved with about 800 mg/d. Evidence from a randomized trial that calcium supplementation modestly reduces colon adenoma recurrence contributes important evidence of causality to the epidemiological studies *(169)*.

Although recommended calcium intakes can be achieved by a high consumption of greens and certain other vegetables, greatly increased intakes would be required for most women to achieve currently recommended levels through the diet without regular use of milk and other dairy products. Calcium supplements are an inexpensive form of calcium that do not include the accompanying calories or saturated fat. Therefore, dairy product consumption can be considered an optional, rather than a necessary, dietary component. Enthusiasm regarding high dairy consumption should also be tempered by the suggestion of many studies that this is associated with increased risks of ovarian and prostate cancer *(170,171)*. The issue of whether an increased risk results from the calcium, lactose, or endogenous hormones in milk remains unclear.

3.8. Salt and Processed Meats

Reduction of salt (sodium chloride) intake from an average of approx 8 to 10 g/d to less than 6 g/d will, on average, decrease blood pressure to a small degree. In a recent review, Law et al. *(172)* concluded that a reduction of 3 g/d would reduce the incidences of stroke and CHD by 22 and 16%, respectively. Although the decrease in risk of cardiovascular disease (CVD) achieved by reducing salt consumption is small for most individuals, the overall number of potentially avoided deaths is large, supporting policies to reduce the salt consumption—particularly in processed foods and by institutions.

In a number of case–control studies, the consumption of salty and pickled foods has been associated with stomach cancer *(173)*. However, because this cancer is relatively rare in the United States, further benefit from reducing salt intake would be small.

4. BODY WEIGHT

Until recently, the issue of optimal body weight was controversial because of analyses that did not account for confounding influences from factors such as smoking (which is a strong cause of premature death and is also associated with low body weight) or for the fact that many individuals, particularly at older ages, have low body weights because of chronic illness *(174,175)*. More detailed analyses indicate that middle-aged persons of even average weight have a high prevalence of abnormalities of blood glucose, lipids, and blood pressure *(176)* and experience substantial increases in MI *(177,178)*, diabetes *(179)*, hypertension *(180)*, many cancers *(181,182)*, gallstones *(183)*, and total mortality rates *(184,185)* compared to their leaner counterparts. Therefore, the current guidelines based on a body mass index range of 19 to 25 kg/m^2 are probably closer to optimal, and the best health experience is achieved by avoiding

increases in weight during adulthood *(174)*. As noted earlier, dietary fat composition over a wide range appears to have little relationship with weight maintenance; in contrast, regular exercise and avoidance of extreme inactivity (e.g., excessive television watching) is crucial *(186)*.

5. ALCOHOL

Many adverse influences of heavy alcohol consumption are well-recognized, but moderate consumption has both beneficial and harmful effects; this combination of effects greatly complicates decisions for individuals (*see* Chapter 33). Overwhelming epidemiological data indicate that moderate consumption reduces risk of MI *(187,188)*, and one to two drinks a day decrease risk by approx 30 to 40%. Although it has been suggested that this effect may be a result of antioxidants in red wine *(189)*, similar protective effects for equivalent amounts of alcohol have been observed for all types of alcoholic beverages *(190,191)*. On the other hand, modest positive associations with risk of breast cancer incidence have been observed in more than 30 studies *(192–194)* for similar levels of alcohol intake, possibly because alcohol appears to increase endogenous estrogen levels *(195,196)*. The overall effect of alcohol (as represented by total mortality) appears beneficial for up to about two drinks per day in men *(197)*. Overall, a similar relationship with total mortality is demonstrated among women, but no net benefit was observed among those at low risk of CHD because of younger age or lack of coronary risk factors *(198)*. Furthermore, the risk of transition from moderate alcohol consumption to addiction and uncontrolled drinking has not been well-quantified.

6. VITAMIN SUPPLEMENTS

Because populations can be identified with very low rates of almost every major disease, vitamin supplements may not further enhance health among populations consuming ideal diets. Only recently have data emerged that address the effects of vitamin supplements against the background of actual diets in the United States, which appear to be far from ideal *(112)*.

The most firmly established benefit, based on case–control, cohort, and randomized studies, is that folic acid supplements in the amounts contained in multiple vitamins can reduce the risks of NTDs by approx 70% *(128,199)*. This is probably only a sentinel indicator of suboptimal folate intakes, as inverse associations also have been seen with colonic neoplasias *(200)*, and low folate intake, along with suboptimal amounts of vitamin B_6, is likely to contribute to elevated blood homocysteine levels and risk of CVD *(122,123,201)*.

In prospective epidemiological studies, men and women who consumed the highest amounts of vitamin E (mostly from supplements) had an approx 40% lower risk of MI compared to those who consumed low vitamin E intakes *(202,203)*. The maximum reduction in risk appeared to occur at levels of 100 IUs or more per day, which is well above intakes achievable by diet alone. However, in randomized trials, mainly among patients with existing CHD, little benefit has been seen *(204)*. The apparent difference may relate to the study populations, because persons with existing CHD were excluded from the epidemiological studies, and they were typically on many drugs that could overlap in mechanisms with vitamin E. In one case–control study, use of vitamin E supplements was associated with a 50% reduced risk of oral cancer *(205)*.

Vitamin C supplementation was associated with lower risk of CHD in one international comparison *(206)*, but the available data did not distinguish vitamin C from other supplements. The association between vitamin C and CHD risk has been inconsistent in other prospective studies *(203,207)*. Apart from a possible reduction in risk of cataracts *(134)*, only limited evidence exists at present that high doses of vitamin C have substantial benefits.

No overall benefit of supplemental vitamin A was observed in a large prospective study of breast cancer *(208)*. Higher intake of preformed vitamin A (retinol) was associated with an excessive risk of hip fracture in prospective studies *(209,210)*, possibly by competing with vitamin D at the receptor level, and elevated risks were seen for use of both multiple vitamins and specific supplements of vitamin A. Serum levels of retinyl esters have not been associated with bone mineral density *(211)*, but these findings are difficult to interpret because retinyl esters are highly variable, and the degree to which a single measure represents long-term vitamin A intake is unclear.

In a randomized trial conducted in a region of China with low consumption of fruits and vegetables, a supplement containing β-carotene, vitamin E, and selenium reduced incidence of stomach cancer *(212)*.

Although far from complete, current evidence suggests that supplements of folate and possibly other vitamins at the RDA level (contained in most nonprescription multivitamin preparations) may have substantial benefits at least for an important, but unidentified, subgroup of the US population, perhaps characterized by increased requirements as well as by suboptimal diets. Because intakes of folate as well as other micronutrients appear marginal for many Americans *(104, 112)*, the risks of using multivitamins appear low, and the cost of supplements is low (especially compared to that of fresh fruits and vegetables), the use of a daily multiple vitamin or one taken several times per week appears rational for the majority of Americans, given current knowledge. A similar argument can be made for vitamin E supplements at levels above the RDA (15 IU), although benefits do not appear to exist for patients with established CHD and further data are highly desirable. For other vitamins and minerals, there presently is limited evidence of benefit of supplements above the RDA levels. Intake of vitamins A and D at levels substantially above the RDA can be potentially harmful, but the safe intake of vitamin D appears to be higher than once believed *(213)*. In one study, intake of supplements containing more than 10,000 IU of preformed vitamin A per day was associated with risk of specific birth defects *(214)*. Therefore, women planning pregnancy should be counseled to take multiple vitamins with folic acid that do not contain vitamin A above the RDA level (8000 IU/d) *(215)*.

7. RECOMMENDATIONS

Any set of dietary recommendations must be made with the clear qualification that information is currently incomplete and conclusions are subject to change with new data. Most of the major causes of morbidity and mortality in the United States have developed over many decades, and large-scale nutritional epidemiological studies have only begun in the last 25 yr; a full picture of the relationship between diet and disease requires additional decades of careful investigation. Nevertheless, combining metabolic, clinical, and epidemiological evidence, the following is a list of several general recommendations that

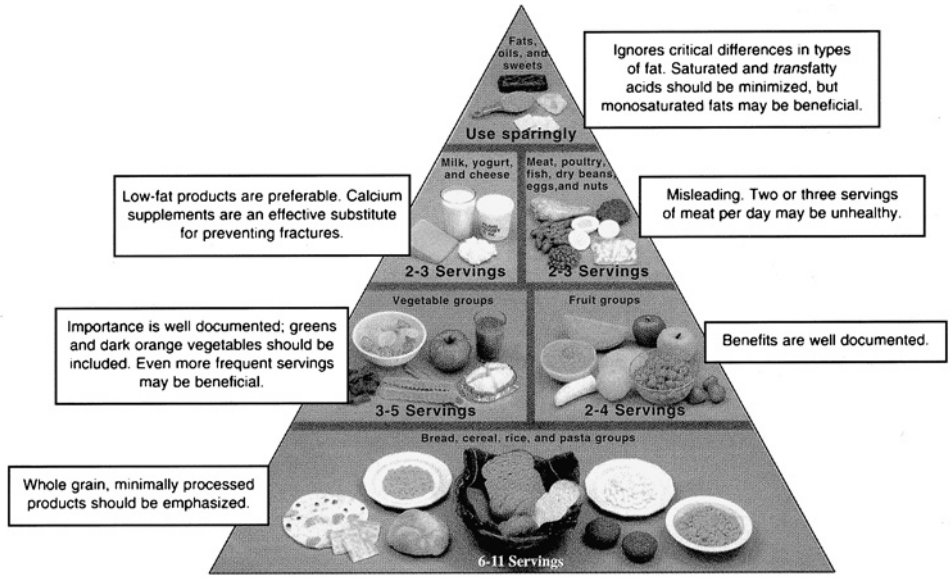

Fig. 1. USDA food pyramid, annotated. Optimal food intakes for children and pregnant or lactating women may need further consideration. (Modified figure reproduced with permission of Nasco Nutrition Teaching Aids.)

are unlikely to change substantially for those who are interested in consuming a healthy diet (*see* Fig. 1):

1. Stay lean and active throughout life. For most individuals, body weight should not increase by more than 5 to 10 pounds after age 21 yr. Because most of us work at sedentary jobs, weight control usually requires conscious regular daily exercise as well as some effort to avoid overconsumption of calories.
2. *Trans*-fatty acids from partially hydrogenated vegetable oils should be avoided completely, and intake of dairy and red meat fat should be consumed sparingly. Unless explicitly stated otherwise, it is safest to assume that deep-fried fast foods and most commercially prepared foods contain *trans*-fatty acids. These unhealthy fats can be replaced with a combination of vegetable oils that include a mix of monounsaturated and polyunsaturated fats. These should include good sources of ω-3 fatty acids, such as canola (rapeseed) or soybean oil.
3. Grains should be consumed primarily in a minimally refined, whole grain form, and intake of simple sugars should be low.
4. Vegetables and fruits should be consumed in abundance (5 servings per day is minimal) and should include green leafy and orange vegetables daily.
5. Red meat should be consumed only occasionally and in low amounts, if at all; nuts and legumes as well as poultry and fish (in moderation) are healthy alternatives.
6. The optimal consumption of dairy products and calcium intake is not known, and dairy products should be considered as optional. High consumption of milk (e.g., more than 2 servings per day) is not likely to be beneficial for middle-aged and older adults and may increase risk of prostate and ovarian cancer. Adequate calcium intake may be particularly important for growing children, adolescents, and lactating women; supplements should be considered if dietary sources are low.

7. Unless one is extremely careful about a healthy food selection at every meal, consuming a daily RDA-level (DV) multivitamin that contains folic acid provides a sensible nutritional safety net. Definitive evidence exists that use of a folic acid-containing multivitamin supplement during the early weeks of pregnancy can prevent a large fraction of NTDs, and the Public Health Service recommends that women of childbearing age consume 400 μg of synthetic folic acid daily—the level found in most multivitamin supplements *(216)*. For most people, this level of intake is difficult and unreliable to achieve by diet alone. As noted earlier, benefits of folic acid supplementation may extend to prevention of MI, stroke, and colon cancer, although this is not proven. Because menstrual losses of iron may not be adequately replaced by iron intake from the low-energy diets of women in a sedentary society, it makes sense for most premenopausal women to use a multivitamin that also contains iron. Because of evidence regarding fracture risk, the amount of preformed vitamin A (retinol) in a multiple vitamin should not exceed the Institute of Medicine RDA of 3000 IU/d, and consuming part of this as β-carotene would be prudent. Pending further data, the use of a vitamin E supplement at a dose of 400 to 800 IU/d is reasonable for most middle-aged and older healthy persons because available evidence suggests that this may reduce risk of MI. Although not proven, evidence also suggests that use of vitamin C supplements at the level of 300 to 500 mg/d may reduce risk of cataract, which may be sufficient reason for some individuals to consider using this supplement. Although there is no documented harm from these supplements at the levels of intake suggested, further studies need to examine long-term effects. Also, personal physicians should be made aware of any nutritional supplements that are being consumed in the event of possible interactions with medications or diagnostic tests. Furthermore, use of supplements should not be considered as an alternative to eating a healthy diet, because foods contain a wide variety of additional factors that are likely to contribute to good health.
8. Finally, be adventuresome in eating! Unfortunately, most of the US population has inherited to the rather monotonous northern European dietary tradition, which is centered on the consumption of meat, dairy products, and potatoes. Contemporary food processing has added to the deleterious effects of this diet by the removal of dietary fiber and micronutrients through over-refining of foods and has profoundly and adversely altered the biological effects of vegetable oils through the process of partial hydrogenation. To further aggravate matters, the worst aspects of diet tend to be the most heavily marketed and promoted. Fortunately, healthy diets do not have to be invented or discovered through new technological advances. Existing foods, together with the lessons of various cultural models of eating based primarily around minimally processed foods from plant sources, provide a means of achieving a diet that is both healthy as well as interesting and enjoyable.

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29 Nutrition and Food Policy in Norway

Effects on Reduction of Coronary Heart Disease

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KEY POINTS

- The Norwegian Nutrition and Food Policy has its roots in the 1930s. A National Nutrition Council (NNC) was established in 1937 and re-organized in1946. Since then, it has had a mandate to give advice to the government regarding nutrition.
- After 1950, there was a substantial increase in mortality from coronary heart diseases (CHDs) that was associated with changes in food habits and lifestyle. In 1963, this increase resulted in an official Norwegian report on the relationship between dietary fat and cardiovascular disease. The report formed a basis for subsequent work, with the formulation of an official integrated Norwegian Nutrition and Food Policy.
- A White Paper was presented to the Storting (Norwegian Parliament) through Report No. 32 (1975–1976) "On Norwegian Nutrition and Food Policy." In 1981, the government presented a follow-up White Paper. Later, the Nutrition and Food Policy was integrated into the health policy in reports to the Storting in 1993 and 2003.
- These four White Papers define the nutritional goals to be achieved for Norway and the measures that the government intends to employ to improve the Norwegian diet.
- The most recent White Paper advocated a broad health policy. It drew attention to the connections between the individual's and the community's responsibility for and possibility of influencing the health situation. At the ministerial level, two official bodies are given the responsibility to coordinate the implementation of the Nutrition and Food Policy: the National Food Control Authority and the NNC.
- The official nutrition and food policy White Papers have been central political and strategic documents in the efforts to improve public health in Norway during the last 30 yr. Currently, we can see several changes in Norwegian eating habits. Fat consumption has decreased from 40 to 34% of the food energy, mainly by a reduction of saturated and *trans* fat. Concomitantly, blood cholesterol has decreased, and mortality from CHD among middle-aged individuals has decreased by 60% during the last 30 yr.
- However, the prevalence of cancer, obesity, and type 2 diabetes has increased in the Norwegian population. The most recent White Paper was seriously concerned with

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these problems and provided suggestions and measures both for the society and the individual for improving the diet and increasing physical activity in daily life during the whole lifetime.

1. THE EARLY HISTORY OF THE NORWEGIAN NUTRITION AND FOOD POLICY

Nutrition and Food Policy has a relatively long history in Norway. It began around 1930 when an official school breakfast was introduced for all children in primary schools *(1)*. Meanwhile, the Norwegian Medical Association took the initiative to study the inter-relation between income, nutrition, and health *(2)*. This issue created an intense debate in the society, and both medical doctors and leading economists contributed to the discussion. It is interesting that prominent economists, two of whom *(3)* later received Nobel Prizes, published papers on diet, nutrition, and income *(3,4)*. Simultaneously, the League of Nations raised the issue that it should be a collective duty of a society to take on the responsibility for a nutrition and food policy. In Norway, the Director General of Health, Karl Evang, strongly subscribed to that idea, arguing that the question of the diet of the people was a national responsibility and demanding solutions through appropriate measures in many sectors in the society. The idea of disease prevention and health promotion as intersectorial work has since gained support in Norway and is now a platform in the health policy. The establishment of a National Nutrition Council (NNC) in Norway in 1937 was closely related to suggestion by the League of Nations. A specific task of the NNC was to create a nutrition and food policy that the government and Parliament could adopt.

The nutritional problems in Norway at that time were caused by poverty, and the measures in the nutrition policy were closely linked to stimulate economic growth and equal distribution of goods and services. Nutritional education, information, and communication also were considered very important.

In 1939, Norway took a major initiative to improve the nutritional situation of the population with the participation of the Ministries of Health, Agriculture, Fishery, and Trade. This initiative was mainly taken to prepare the population for difficulties if Norway were to be involved in the war in Europe. Norway was involved in the war and was occupied by Germany for 5 yr.

During this occupation, the national food supplies were scarce. However, few negative nutritional effects on health were observed in the adult population; in fact, the mortality from coronary heart disease (CHD) decreased *(5)*.

The idea behind the establishment of the Food and Agricultural Organization of the United Nations (FAO) in 1945 was a "marriage" between food production and health promotion through better nutrition. FAO requested each of its member nations to establish a nutrition council that could prepare and coordinate implementation of an intersectorial food and nutrition policy. Evang played an important role in the planning of the FAO, and in 1946, he re-organized the NNC. The new NNC was established within the Ministry of Health and Social Affairs. In some other FAO member countries, similar councils were established within the Ministries of Agriculture.

Since its reorganization in 1946, the NNC has always had a mandate to give advise to the government concerning nutrition and dietary issues (i.e., to formulate recommended dietary allowances and guidelines on fortification of food with vitamins and other micronutrients). The fortification policy has always been very restrictive.

Until about 1950, the main challenge for the Norwegian Nutrition and Food Policy was to ensure enough food for the population. After 1950, however, the dietary problems changed in character. The country's economy improved significantly, and the market flourished with foods. People could eat the formerly recommended energy-rich food in abundance, with no knowledge or warning that these foods could create health problems. However, problems connected with overnutrition and unbalanced diet became more prevalent. There was a substantial increase in mortality from CHDs *(6)*, an increase that was associated with changes in food habits and lifestyle. The responsibility for the society to address this problem became more obvious. The Director General of Health established an expert advisory committee. This resulted in an official Norwegian report on the relationship between dietary fat and cardiovascular disease (CVD) in 1963. The experts recommended a decrease in the fat content of the diet to 30% of the dietary energy, mainly through reduction of the intake of saturated fat *(7)*. The report formed a basis for subsequent work (i.e., the formulation of an official integrated Norwegian Nutrition and Food Policy). However, it took quite some time before this new nutritional challenge became an important target in an official nutrition and food policy, mainly because of a resistance from the agriculture and dairy industry.

2. THE STRUGGLE FOR INTRODUCING NEW RECOMMENDATIONS IN GOVERNMENT POLICIES

As the suggested relationship between diet and CHDs gradually gained acceptance, the NNC made several attempts to influence government policy goals to align with the new knowledge. The major effort was a suggestion to the Directorate of Health in 1964 to prepare a White Paper on nutrition policy. The proposal gained no political support.

Toward the end of the 1960s, the NNC redefined its approach to a more politically oriented strategy, seeking support among members of the Parliament. By lobbying, the NNC persuaded members of the Parliament to use their opportunities to directly question ministers (Cabinet members) in the Parliament about diet and health. Also, consumer interests and concern with food and health were growing. This brought nutritional issues to the attention of not only the members of the Parliament and ministers but also the media and the general public.

These efforts brought considerable attention to the issues of nutrition, health, and agriculture. However, they were not sufficient in creating actual changes at a policy level. Until 1973, the main actors responsible for the nutrition policy, the Ministry of Social Affairs and the Directorate of Health, were still rather passive and made no explicit effort to bring about policy changes. Parallel to the increased attention on nutritional issues, a concern regarding agricultural policy was growing both at the political and public levels. There was fear that the support for the most peripheral rural areas would decrease due to the economic cost of having agricultural production in remote areas.

3. GOVERNMENTAL WHITE PAPERS ON NUTRITION AND FOOD POLICY

At the FAO/World Health Organization (WHO) World Food Conference in Rome in 1974, it was again stated that each nation should have a nutrition and food policy. The

Norwegian delegation at the conference was headed by the Minister of Agriculture, who stated in his main address to the conference that the industrialized countries should also formulate a nutrition policy. When the minister returned to Norway, he took the initiative to form a Norwegian Nutrition and Food Policy. A White Paper was presented to the Parliament through Report No. 32 (1975–1976) "On Norwegian Nutrition and Food Policy" from the Ministry of Agriculture *(8)*. To the best of our knowledge, this was the first official White Paper in the world regarding an integrated Nutrition and Food Policy.

The goals and considerations that this policy was supposed to coordinate may be summed up in the following four major objectives:

1. Healthy dietary habits should be encouraged.
2. A nutrition and food policy should be formulated in accordance with the recommendations of the World Food Conference.
3. The policy should aim at increased production and consumption of domestic food and at strengthening the ability to rapidly increase the degree of self-sufficiency in the food supply.
4. As a result of regional policy, the highest priority should be given to using the food production resources in the economically weaker areas.

Furthermore, it was stated that a primary task of the Nutrition and Food Policy was considered to be an active coordination of these four major goals.

3.1. Objective 1: Encourage Healthy Dietary Habits

In 1975, the government said that:

"The relationship between diet and health, for instance between diet and cardiovascular diseases, is not yet entirely understood. However, there is sufficient knowledge of this relationship to recommend alterations in the diet which are desirable from the point of view of preventing these diseases...

The Government will prepare a policy which will contribute to the following:

- The beneficial aspects of the diet are to be preserved. Conditions will be arranged so that the diet is better adapted to nutritional requirements, while general demands for taste, variety and diversity are stressed. The different nutritional requirements of special groups such as children and young people, pregnant and breastfeeding mothers, the elderly, etc. must also be taken into consideration.
- In order to obtain a better adaptation of the diet to nutritional requirements it is especially important to curtail the proportion of fat in the energy supply. An objective should be to reduce the proportion of fat to 35 % of the energy supply through a gradual alteration of the diet.
- The decrease in the supply of fat should be replaced by foods containing starch—primarily cereals and potatoes. There should be an attempt to limit the proportion of sugar in the energy supply.
- The proportion of polyunsaturated fatty acids in the total fat intake should be increased."

It should be noted that the goal for the average fat consumption in the policy document was 35% of the energy intake, whereas the NNC had advocated for 30% of energy intake. The official goal was a compromise between the NNC and the agricultural lobby, which feared that a lower fat target would lead to a reduced consumption of meat and dairy products.

In this first White Paper, it was suggested that the central issue was which means should be taken to influence the production and consumption of food products in accordance with the goals and objectives of the policy. Emphasis was put on collaboration between the public sector; the organizations, enterprises, and employees in the relevant economic sectors; the voluntary organizations; and the various categories of households. The information/education/training measures were particularly emphasized, aiming at motivating people to adopt better dietary habits, securing a broad general knowledge of the main principles of a nutritionally healthy diet, and providing the possibility to acquire the necessary skills.

Therefore, the means used have addressed a wide range of areas, such as educating and informing the public and professional arenas (at universities, for teachers, adult education, mass media, consumer organization media, conferences and meetings with primary schools and professional groups); setting consumer and producer price and income subsidies together in nutritionally justifiable ways; adjusting absolute and relative consumer food price subsidies; guaranteeing low prices for food grain, skimmed and low fat milk, vegetables, and potatoes; avoiding low prices for sugar, butter, and margarine; marking regulations to promote provision of healthy foods by retail stores, street vendors, and institutions; and regulating food processing and labeling *(8,9)*.

The NNC was responsible for the follow-up of the National Nutrition and Food Policy. It did not have any decentralized organization to instruct, and funds and staff were limited. In implementing activities, cooperation with other sectors and organizations was important. NNC took responsibility for contact with the school system, the health sector, the local food control authority, the consumer organizations, nongovernmental organizations (NGOs)/voluntary organizations, the food industry, food retailers and wholesalers, and mass media.

The NNC had regular meetings with major companies in the food industry, organizations dealing with restaurants and institutional catering, politicians, and the mass media. The NNC focused on providing services (such as printed material), giving lectures, arranging seminars, and stimulating activities by providing funds for information and public education campaigns and new methods for health promotion at the municipal level.

One of the successes of the Norwegian National Nutrition and Food Policy was the information and public education performed by the NNC, especially by the use of programs on the national public TV and nationwide, longlasting nutrition campaigns.

Higher training or education at university level was also important. The University of Oslo has trained nutritionists since 1965. They form a cadre of professionals who are working in many different organizations and at different levels and are key in the implementation of many nutrition-related activities.

The development at the Medical Faculty at the University of Oslo was also important, because in the early 1970s, nutrition education there was virtually nonexistent. Nutrition as a subject developed first as an elective and later as a compulsory course in which students were exposed to nutrition as an area of applied biochemistry and physiology, with some lectures that included diet, clinical nutrition, and nutrition policy. Teachers instructing the course also produced a tailor-made text book for nutrition in medical training *(10)*. The wide contacts that medical doctors have with the public, their role in preventive and curative health work, and the status they have in the minds of the public make them important communicators of nutrition.

Two other projects in the health sector were of great importance for the implementation of the nutrition policy. First, a randomized prospective study called the "Oslo Study" showed that a change in diet reduced CHD in middle-aged men with initially high blood cholesterol levels *(11)*. The results of the Oslo Study convinced both the population and the politicians about the importance of changing to a more lean diet. Second, the National Health Screening Service conducts health surveys in all areas of Norway *(12)*. All inhabitants age 40 yr are offered a health examination that includes measurement of blood lipids, blood pressure, and chest X-ray. Persons with high risk factors for CHD are referred to the local health authorities for follow-up. Until recently, the health surveys have been done at intervals of a few years; they have provided valuable information on CHD risk factors, body mass index, and other health parameters in different parts of Norway and have documented changes in these parameters during the period that the country has had an active nutrition policy *(13)*. These surveys are now being re-organized.

3.2. Objective 2: Formulate a Nutrition and Food Policy in Accordance With the Recommendations of the World Food Conference

In 1975, the government said:

> *"Taking the recommendations from the World Food Conference as a starting point, it would be right to view the nutrition and food policy in relation to the global situation as regards production and supplies. As part of solving the world's food problems, the developed countries were recommended to increase their food production and to implement measures for improving their own pattern of consumption. Such measures must, however, make allowances for the developing countries' trade interests as promoted through international organizations."*

The means used to achieve this include earmarking emergency grain in Norway, providing aid and support to poor countries, supporting United Nations agencies, and contributing to the World Food Program *(9)*.

3.3. Objective 3: Increase Production and Consumptionof Domestic Food and Strengthen the Ability to Rapidly Increase Degree of Self-Sufficiency in the Food Supply and Objective 4: Give High Priority to Using Food Production Resources in Economically Weaker Areas

The Norwegian government encouraged farmers to increase production of food grain, potatoes, vegetables, and low-fat milk. Measures were introduced to stabilize milk and meat production to avoid overproduction. In collaboration with farmer's organizations, an attempt was made to produce more grass-fed, rather than grain-fed, beef. Furthermore, the pork produced was less fatty than before.

There also has been an integration of support, such as support to fisheries and to fodder production for dairy cattle in northern mountain areas and in remote regions; encouragement of wheat production and discouragement of dairy production in the southern part of the country; provision of transport and other subsidies to retailers in outlying regions; and an integration of agriculture and food policy with other economic planning initiatives.

The link between the food policy and district policy is a reflection of an official policy in Norway that has been in place for the last 50 yr: support to remote areas of the country

to diminish migration to centers and cities. This district policy was also a part of the Norwegian defense policy during the Cold War.

4. RESISTANCE AGAINST THE NUTRITION AND FOOD POLICY

The progress in implementing the nutrition policy was slow during the first years after its approval by the Parliament in 1976. Two main reasons were that the diary and meat industry were against the policy and that the NNC had little power and political influence.

The diary industry tried to counteract the policy by inviting foreign experts who claimed that milk, butter, and other dairy products had no influence on risk factors for CHD and, therefore, the Norwegian nutrition policy was built on false premises. This led to a survey among leading scientists in the field of atherosclerosis and lipid metabolism *(14)*. The survey clearly showed that the Norwegian National Nutrition and Food Policy was built on a solid scientific basis. (Interestingly, this survey came to play an important role in the US Congressional hearings on March 23, 1977, regarding McGovern's document on dietary goals for the United States.)

The NNC tried to push the diary industry to produce low-fat milk for the Norwegian market. But the dairy industry resisted. However, after the NNC organized a successful consumer-based campaign for consumption of skimmed milk instead of whole milk, the dairy industry relented, and, in collaboration with the NNC, low-fat milk was introduced in 1984. Since then, there has mainly been good collaboration between the NNC and the dairy industry, which have gradually produced several low-fat dairy products.

The Norwegian Nutrition and Food Policy also has internal conflicts. Therefore, the objective to increase local food production led to more Norwegian wheat for human consumption. However, Norwegian grain was more expensive than wheat on the international market, and, simultaneously with an official campaign to eat more cereals, the bread got more expensive.

The first White Paper on the National Nutrition and Food Policy gave the task of coordinating the implementation of the nutrition policy to two official agencies: the NNC and the Inter-Ministerial Coordinating Committee on Nutrition (IMC). The latter was formed to coordinate all political sectors involved in the National Nutrition and Food Policy. The IMC was supposed to be chaired by the Deputy Minister of Health and Social Affairs and was to consist of leading civil servants from nine ministries. However, the IMC was never formed as it was planned, probably because of competing priorities among the different ministries. At first, the inactivity of IMC also deactivated the NNC, rendering it a body with a lot of good intentions but with little power and political influence. Later, however, because of the inactivity of the IMC, the NNC took direct political contact with the government and members of the Parliament. Thus, NNC gradually gained both power and political influence.

In 1981, the government presented a follow-up White Paper: Report No. 11 (1981–1982) "On the Follow-Up of Norwegian Nutrition Policy" *(15)*. The Ministry of Health and Social Affairs was responsible for this report. Note that the word "Food" was removed from the title. This was a sign that the main issue of the policy was more health and less agricultural policy.

The Nutrition and Food Policy was integrated into the health policy in Report No. 37 to the Storting (Norwegian Parliament; 1992–1993), "Challenges in Health Promotion and Prevention Strategies," *(16)* and Report No. 16 (2002–2003), "Prescriptions for a Healthier Norway—A Broad Policy for Public Health" *(17)*.

These four White Papers have defined and still define the nutritional goals for Norway and the measures that the government intends to employ to improve the Norwegian diet. All four reports underline that dietary changes should be voluntary.

5. THE CURRENT NUTRITION AND FOOD POLICY IN NORWAY

The goals for the Norwegian Food and Nutrition Policy that were established in 1992 are still valid today. The current Norwegian Nutrition and Food Policy has the following four main goals:

1. Reduce the prevalence of dietary-related diseases and to achieve the following objectives:
   - A national diet in line with the recommendations of the NNC.
   - Breastfeeding of babies should continue at a high level.
   - The nutrition-related differences in health status in the population should be reduced.
   - Dietary treatment should be integrated in primary and secondary prevention of diet-related diseases within the health services.

   The goals deal with the national diet, including that of the infants; the present inequality in health, especially CVDs; and mechanisms to change approaches within the health services. Food consumption should fulfill the following nutritional goals:

   - Reduce the proportion hard fats (the sum of saturated and *trans* fats) to about 10% of energy intake and to reduce the total fat in the diet to about 30% of energy intake.
   - The fiber content of the diet should be increased to 25 g per person per day by increasing the consumption of cereals, potatoes, vegetables, and fruit.
   - Sugar should contribute no more than 10% of the energy.
   - Salt intake should be reduced to 5 g per person per day.
   - Increased consumption of fish.
   - A change in dairy products, meat, and meat products in favor of those with lower fat content.
   - The consumption of butter, hard margarine, high-fat potato products, and snacks should be reduced.
   - A frequent intake of grilled, fried, smoked, or salted foods should be avoided.
2. Ensure food safety. The policy shall facilitate the following objectives:
   - Foods should be free of infectious agents and should not contain approved food additives in quantities that are a risk to health.
   - Consumer demands for food quality, safety, and security should be met.
3. Strengthen consumers' influence on the Nutrition and Food Policy, the government will ensure that the consumers have a real possibility of providing themselves with a health-promoting diet. To maintain this, a consumer policy that stimulates a health-promoting diet through differential prices, food availability, food labeling/claims, and information and marketing will be implemented.
4. Contribute to safe production, distribution, and marketing of food products and to safe consumption patterns regarding health, the environment, and appropriate use of resources. Food production shall satisfy society's requirements for safe and ethical acceptable norms and allow for protection of animals and plants, genetic resources, and environmental resources. This aspect of the National Nutrition and Food Policy

> is an adjustment to sustainable development. Therefore, it is appropriate to quote from The Brundtland Commission: "The industrialized countries must reduce overproduction of agricultural products, so that world market prices will reflect the real production costs. In the short term this implies higher costs for food-importing from developing countries. But it will mean a real stimulus to increase production in developing countries" *(18)*.

The White Paper of 2003 *(17)* gives even more emphasis on health promotion and disease prevention. This White Paper draws up strategies for a healthier Norway.

There are several reasons that 10 yr after the Storting debated the White Paper No. 37 (1992–1993) Challenges in health-promoting and preventive work, the Norwegian Government submitted a new White Paper that contained strategies for the next 10-yr period. New trends in society bring new health challenges. The latest White Paper is called "Prescriptions for a Healthier Norway. A Broad Policy for Public Health." Some of the reasons for a new White Paper include globalization, a multicultural society, a demanding workplace, and drug and alcohol problems. The WHO has shown that one-third of the total burden of disease in industrialized countries is caused by five risk factors: tobacco, alcohol, high blood pressure, cholesterol, and excess weight *(19)*.

Against this background, the government wants to revitalize public health work. Its objective is a healthier Norway through a policy that contributes to more years of healthy life for the population as a whole and a reduction in health disparities between social classes, ethnic groups, and the genders.

This new White Paper aims for a more systematic and holistic policy than what existed before. The government attaches importance to the connection between the community's and the individual's responsibilities for, and possibility of, influencing the health situation and to showing what the individual and the community can gain from effective preventive health care. The government calls for a national public health chain, which will also provide a basis for a far more systematic cooperation with voluntary organizations, educational institutions and other bodies. Work must also be done to further develop and reinforce preventive work as a central instrument within the health services. Additionally, the focus will be on ensuring that the measures implemented are based on experience and new knowledge acquired, for example, from research and pilot projects. A better understanding of complex causal relations will provide more effective measures.

The new White Paper advocates a broad health policy. It addresses the many factors that play a part in creating health problems and that help to protect us from disease. It seeks to draw attention to the connections between the individual's and the community's responsibilities for, and possibility of, influencing the health situation and it attempts to make responsibility visible in a number of sectors and policy areas.

The government gives particular emphasis to the following general strategies for public health:

- Make it easier for people to take responsibility for their own health.
- Build alliances to promote public health.
- Encourage more prevention, rather than cure, in the health service.
- Build new knowledge.

6. GOVERNMENTAL IMPLEMENTATION BODIES

At the ministerial level, the responsibilities to coordinate the implementation of the National Nutrition and Food Policy are given to two official bodies: the National Food Control Authority and the NNC. The IMC mentioned earlier did not fulfill its intentions and has been abandoned from the current nutrition policy.

The National Food Control Authority was established in 1988 to administer legislation related to food. It is responsible for coordinating and guiding the executive control system, which consists of 89 Local Food Control Authorities covering all municipalities in Norway. The local food control is responsible for inspecting production, processing, import, storage, and transport of food. The National and the Local Food Control Authorities have, in cooperation with the NNC, involved themselves in a broader nutritional work (e.g., in schools and catering). In 2004, The National Food Control Authority joined with several other organizations and was re-organized into a new Norwegian Food Safety Authority.

The NNC was established in 1937 and re-organized in 1946. The appointed members and organization of the NNC have changed over time. During 1999 to 2003, it was named the National Council on Nutrition and Physical Activity. Today, two separate councils (National Council on Physical Activity and NNC) are appointed for the period of 2003 to 2007. The NNC is currently an expert body that consists of 15 members with competence in nutritional biology, clinical nutrition, epidemiology, public nutrition, and nutrition policy. The NNC cooperates closely with research institutions. Main features in the mandate for the council are:

- Act as an advisory body to ministries and others in matters concerning food supply and nutrition.
- Describe and evaluate the nutritional situation in Norway.
- Propose new strategies and measures to reach nutritional goals.
- Provide assistance and proposals to research councils and research institutions.
- Contribute to the promotion and coordination of professional work on diet and nutrition, both nationally and internationally.
- Be actively engaged in nutrition education and ensure that public information on nutrition is in accordance with recommendations.

One important task for the NNC is the development and revision of the Norwegian Dietary Guidelines. These guidelines were first published in 1954 and have been the theoretical basis for nutrition-related health promotion and incorporated as the scientific basis in the National Nutrition and Food Policy. Since 1980, these guidelines have been prepared in cooperation with the other Nordic countries (the latest preparation occurred in 1996) *(20)*.

During the 1990s, the administration of the NNC was strengthened from being a small secretariat to an efficient administrative body with broad knowledge about nutrition and physical activity, operating in close contact with both the Ministry of Health and the Council and its members. In 2002, the Directorate for Health and Social Affairs was established and the administration of the Council was incorporated in the Directorate as two separate departments for nutrition and physical activity. It has been very important for the NNC to have a strong and competent administration so that it may implement and coordinate nutrition-related activities in many different sectors of the society. An

intersectorial approach is one of the most important elements in the Norwegian National Nutrition and Food Policy.

7. SOME RESULTS OF THE NORWEGIAN NATIONAL NUTRITION AND FOOD POLICY

Nutrition and food policy is clearly a field of conflicting interests. Nevertheless, government policy documents have helped to further cooperation between the various sectors that have an impact on the diet of Norwegians. We can see promising dietary changes (*see* Tables 1 and 2). However, we are still far from fulfilling the goals of the policy.

Dietary data are obtained from the annual reports of the Directorate for Health and Social Affairs *(21)*, which present food consumption data based on wholesale figures, and from the household consumption surveys published by Statistics Norway, which provide figures concerning food entering the household. Although figures are somewhat uncertain for some food groups (i.e., fish, vegetables, and fruits), they provide a basis on which to draw certain conclusions regarding development and trends in food consumption during the last 20 yr (Table 1).

The consumption of cereal products showed a positive trend until the beginning of the 1980s. After a decrease for some years, consumption has increased again. The proportion of cereals of Norwegian origin have risen considerably; flour milled from Norwegian cereals now comprise approx 40 to 50% of total food flour consumption, whereas in 1975, not a single grain produced in Norway was consumed by humans and was only used for animal feed.

The total milk consumption has decreased. From 1970 to 1985, the consumption of skimmed milk rose, and the use of whole milk decreased. After an initiative from the NNC, the Norwegian Dairies introduced partially skimmed milk (low-fat milk) with a fat content of 1.5% into the Norwegian market in 1984. Compared to whole milk and skimmed milk, partially skimmed milk rapidly took a considerable share of the market. In 2000, a low-fat milk (with a fat content of 0.7%) fortified with vitamin D was marketed. The consumption of cream has remained at a stable level, but the consumption of cheese has increased. Milk and other dairy products are still the major sources of saturated fat in the Norwegian diet. The NNC advocated that partially skimmed milk should replace whole milk in school milk programs. In 1984, most of the school milk was whole milk. Soon, most of the whole milk in schools was replaced by low-fat milk and skimmed milk, and in 2002, 86% of the school milk was low-fat milk.

Consumption of vegetables has increased over a long period. Wholesale figures for fruit have not increased, but data from household consumption surveys indicate a 25% increase in fruit consumption since 1975. Norwegian-produced vegetables comprise 60% of total consumption of vegetables, whereas import comprises about 90% of the total amount of fruit consumed. The consumption of potatoes has decreased, and the share of processed potato products is increasing. Potatoes as raw materials for further processing now comprise about 50% of all potato sales compared to 10% in 1975. The sale of potato chips has particularly increased.

Meat consumption has increased considerably. However, the meat produced contains less fat than before. The basis for estimation of fish consumption is uncertain, but estimates were made on the basis of household panel surveys indicating a consumption of about 34 kg per person per year in 2002.

Table 1
Food Consumption at Wholesale Level (kg/Person/Year)

	1970	*1975*	*1980*	*1985*	*1990*	*1995*	*2000*	*2002*
Cereals	71	75	80	75	79	81	85	86
Potatoes, unrefined[a]	79	71	63	63	52	45	34	29
Potatoes, refined[b]	7	8	12	17	19	21	30	32
Vegetables	40	37	46	47	53	58	59	60
Fruits and berries	67	74	76	85	78	79	69	73
Meat	43	52	54	54	53	62	64	67
Egg	10	10	11	13	12	10	10	10
Fish[c]						28	31	34
Whole milk (3.9%)	172	169	160	124	64	45	35	33
Low-fat milk (0.7 + 1.5%)				28	79	85	70	70
Skimmed milk (0.1%)	14	26	26	30	32	26	19	18
Cheese	9	10	12	13	13	14	15	15
Cream	6.7	6.7	6.6	6.7	6.9	6.7	6.6	7.2
Butter	5.4	4.7	5.4	4.8	3.4	3.0	2.8	2.9
Margarine, total	19	18	15	14	13	13	11	10
Low fat margarine	0	0.5	0.2	0.2	1.9	2.6	3.0	3.0
Oil and other fats[d]	4.4	4.2	4.9	4.1	4.0	4.0	4.0	4.0
Sugar, syrup, honey, etc.	42	32	45	42	41	41	42	43

[a]Potatoes not for industrial uses
[b]Potatoes used to produce potato products.
[c]Fish consumption estimated by household panel surveys. Food Balance Sheet figures for fish consumption not available.
[d]Includes cooking oils, cooking fats, and fat used in food manufacturing for mayonnaise, salads, chocolate, biscuits, etc.
(From Directorate for Health and Social Affairs, Oslo, Norway.)

Table 2
Dietary Energy and Energy-Providing Nutrients at Wholesale Level (Person/d)

	1970	*1975*	*1980*	*1985*	*1990*	*1995*	*2000*	*2002*
Energy, kcal	2870	2920	3180	3040	2920	2870	2870	2870
Protein, g	85	87	94	94	94	93	95	95
Fat, g	126	129	135	122	111	115	110	111
Carbohydrate total, g	352	345	390	378	375	361	378	371
Sugar, g	115	91	120	118	118	117	120	122
Dietary fiber, g	—	21	23	22	22	23	24	23
Percentage distribution of energy								
Protein	12	12	12	13	13	13	13	13
Fat	39	40	38	37	35	36	34	34
Carbohydrate, total	49	48	50	51	52	51	52	52
Sugar	16	13	15	16	17	17	17	17

(From Directorate for Health and Social Affairs, Oslo, Norway.)

The consumption of edible fat has dropped steadily since the 1950s. The decrease has resulted from reduced consumption of margarine and butter. The proportion of margarine with a relatively large content of vegetable oils and low-fat margarine has increased. Furthermore, the consumption of edible oils and oil-containing products such as mayonnaise is still increasing.

Calculations based on wholesale figures and consumer consumption studies show that the energy of fat in the diet increased from about 20% in 1900 to 40% in 1975 *(21)*. It currently has decreased to 34% of energy of fat (Table 2).

The dietary fatty acid pattern has improved. Calculations based on household consumption surveys from 1977 to 1979 and 1999 to 2001 showed a decrease in dietary content of *trans* fatty acids from approx 4 to 1% of energy and a decrease in saturated fatty acids from 17 to 14% of energy *(21)*. The dietary content of cholesterol per 1000 kcal increased from below 100 mg (in the beginning of the century) to 180 mg (1950) and later decreased to approx 110 mg per 1000 kcal *(22)*.

For the past 20 yr, sugar has comprised 15 to 17% of the energy content at wholesale level and 14 to 15% of energy intake in household consumption surveys. According to national dietary surveys, adult Norwegians have an intake of 8 to 9% of energy from sugar. Among adolescents, sugar intake has increased from approx 12 to 18% of energy from 1993 to 2000. The annual consumption of soft drinks and sweets has increase several-fold in the last 25 yr. In 2002, Norwegians consumed an average of 91 L of sugar-containing soft drinks *(21)*.

8. IMPORTANT CHARACTERISTICS OF THE NATIONAL NUTRITION AND FOOD POLICY AND ITS IMPLEMENTATION

A basis for the National Nutrition and Food Policy is an agreement regarding the idea that many factors affect the diet and the prevalence of diet-related health problems. The consensus achieved about the goals and the strategies of the Norwegian National Nutrition and Food Policy is of major importance for the implementation of the policy. This has been achieved by forming the goals of the policy on a scientific basis and by stimulating scientists to participate in the implementation of the policy. The Norwegian experience has shown that the content of a strategy intended to influence the diet in a desired direction should define the following:

1. Goals and objectives: Thematically, the objective should be linked to reduced risk of disease, better growth and development, more optimal supply of nutrients, or a healthier diet in general. The target groups should be identified.
2. Strategies and measures: Strategies should be expressed generally and should be linked to the goals and objectives. The measures should be specified as part of each strategy, and may be implemented in a number of arenas. Often, several arenas will be used simultaneously in a total effort to achieve the goals. The best area in which to invest the efforts depends on knowledge of nutrition problems in the population. Epidemiological surveys are required to provide information regarding relevant conditions. It is particularly important to identify groups that may be more susceptible to nutritionally related health problems than the population in general. The monitoring of the Norwegian nutrition and food situation has been strengthened considerably through several nationwide nutrition surveys and screening programs.

It is important to encourage a nutritionally adequate diet through recommending consumption of healthy food. The NNC has worked to influence consumer choices by

information and advice through various communication channels. The goal has been to influence what consumers purchase by, for example, adequate labeling, marketing, and changing the relative prices and the availability of different foods. Cooperation with manufacturers and merchants has been important during this process.

The strategies for disease prevention and health promotion often are grouped into mass strategies, target group strategies, and high-risk group strategies. The mass strategy involves the whole population. Several longlasting nationwide campaigns have been important elements of the mass strategy initiated by the NNC. In these campaigns, the public radio and television frequently have been used to dispense information about how to prepare and consume a healthy and low-fat diet. In target group strategies, the focus has been directed at various groups of the population, such as infants, school children, the elderly, and immigrants. The national high-risk strategies have been based on screening of large groups of the adult population to identify persons at high risk for nutrition-related disorders and then introducing measures directed specifically toward the identified individuals *(12,13)*.

The choices of strategy and measures include identifying arenas for intervention. Relevant arenas include public services, especially the health and social welfare services (curative and preventive), schools, shops, and the mass media. Governmental organizations, employers and employee's organizations, and the various NGOs have been important actors in the work to promote health through a better nutrition. The food industry and the retailers have also been of major importance

9. CONCLUSION AND RECOMMENDATIONS

In our opinion, the official National Nutrition and Food Policy White Papers *(8,15–17)* have been central political and strategic documents in the efforts to improve public health in Norway over the past 30 yr, as documented earlier. The NNC has played an important role in the implementation by developing a consistent and scientific basis for the policy. The NNC has often been consulted in connection with widespread efforts in various sectors (also outside the health sector) and has developed a basis for various political decisions.

We have learned the following about forming and carrying out a nutrition and food policy:

- It takes time to get politicians interested in the issue. To get a comprehensive and long-term policy, the government needs to develop a policy that has broad political support and that is not subject to changes linked to specific governments in power.
- A nutrition and food policy must address both problems related to inequality and lack of food (malnutrition) as well as CVDs and other health problems that are related to too much food, unbalanced diets, and changes in lifestyle (overnutrition).
- The nutrition message to the public must be scientifically based, consistent, credible, and relevant for the target groups of the population. It must be practical and easy to understand.
- An effective administrative body (e.g., a nutrition council) and a strong secretariat are needed so that it is clear who has the daily responsibility to implement the policy.
- The nutrition council must regularly report to the ministry in charge and the Parliament.
- The members of the nutrition council should be independent experts in nutrition, diet, and health with broad experience.
- Cooperation between the nutrition council, NGOs, the food industry, and other key actors is important.

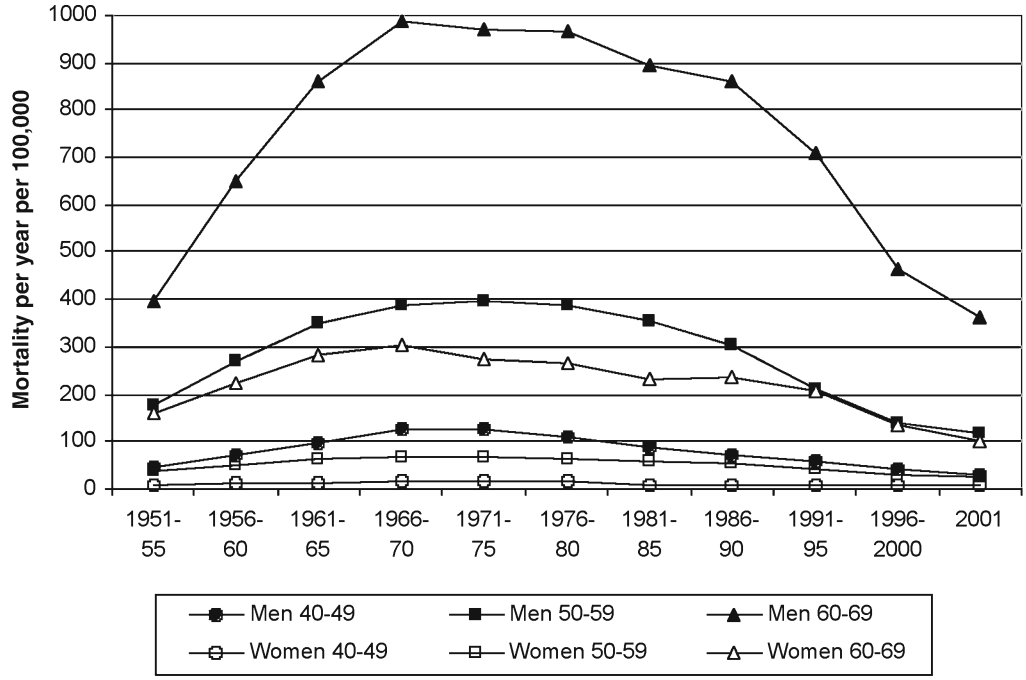

Fig. 1. Mortality from coronary heart disease in Norway. The data are compiled from Norwegian National Bureau of Statistics.

Today, we can see several changes in Norwegian eating habits. The consumption of cereals, vegetables, fruit, and low-fat milk has increased. Furthermore, there has been a reduction in the intake of margarine, butter, and whole milk. This has led to a reduction of total fat from 40 to 34% of the food energy, mainly by a reduction of saturated and *trans* fat.

Concomitantly with these changes, the surveys done by the National Health Screening Service have shown that blood cholesterol has decreased by about 10% in the Norwegian population *(6,13)*. These changes have probably contributed to the 60% reduction in the mortality of CHD among middle-aged men and women during the last 30 yr (Fig. 1). The mortality of CHD among Norwegians is now approaching the level in Greece, but it is still much higher than reported in France and Japan *(23)*.

In the beginning, the nutrition policy in Norway was, to a large extent, aimed at a reduction in premature CHD.

It is most probable that the Norwegian National Nutrition and Food Policy has played an important role in changing dietary habits and has thus contributed to the decline in heart disease. This goal has been obtained to some extent, and, therefore, the current nutrition policy has been changed somewhat compared to what was formulated in 1975. One reason for this is that the prevalence of cancer *(24)*, obesity *(25)*, and type 2 diabetes *(26)* has increased in the population. In fact, the increase in body weight has been so substantial and rapid that we may soon see an increase in CHD, both in men and women. The diet for the Norwegian population has been improved. However, the consumption of energy-dense foods, such as sweets and soft drinks, has increased dramatically. The

increase in excessive weight most likely results from an inability to adjust energy intake to a decreasing level of physical activity within the population. The most recent White Paper *(17)* seriously addresses these problems and provides suggestions and measures both for the society and the individual for increasing physical activity in daily life during the entire lifetime.

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30 Prevention of Malnutrition in Chile

Fernando Mönckeberg Barros

KEY POINTS

- Chile has been successful in reducing infant mortality and malnutrition in recent decades.
- Targeted health, nutrition, and education programs have been key for this reduction.
- Results show that to be successful, a nutrition and health policy must adapt to the socioeconomic strategy of the government.
- Food distributed by health centers has improved attendance of the public to those centers and can be used for other health-related measures.
- School feeding programs have decreased school desertion in Chile.

1. INTRODUCTION

During the last 40 yr, a progressive and continuous improvement in the health and nutrition of infants and preschool children has occurred in Chile. Biomedical indicators show that Chile has reached one of the highest levels in the region, although during the first two decades per capita gross national product (GNP) had not substantially changed. Also during this 40-yr period, economic policies have changed drastically, ranging from a planned, centralized economy, to an open, liberal economy, which has been in place since 1976. In the last 30 yr, the country has confronted many severe economic and political crises. In 1973, a Socialist government was overthrown by a military coup. Two severe economic crises occurred in 1975 and 1982, associated with high unemployment rates that in the latter period reached 20% of the total labor force. Despite these numerous changes and crises, malnutrition and infant and preschool child mortality have continued to decrease.

The situation was quite different prior to the early 1960s *(1)*. Chile had one of the highest infant mortality rates in Latin America (120 per 1000). This decreased to 11 per 1000 in 1994 and 9 per 1000 in 2002, the lowest rate in the region (Fig. 1). This decline in the infant death rate resulted from a remarkable decrease in infant mortality caused

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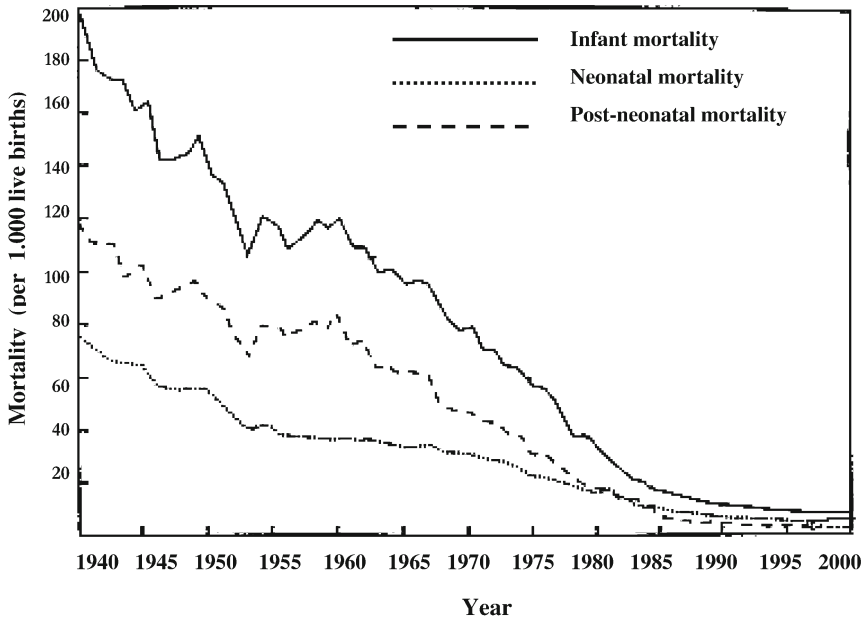

Fig. 1. Decline in infant mortality in Chile from 1940 to 1988. (Source: Ministry of Health, Chile.)

Table 1
Chile: Infant Mortality (Age 0 to 1 Yr) as a Result of Respiratory and/or Diarrheal Disease, 1960 to 2000 (per 100,000 Live Births)

Year	*Respiratory disease*	*Diarrheal disease*
1960	4.318	1.365
1970	2.793	1.476
1980	502	161
1990	86	210
2000	36	12

Source: Informe Anual, 2000 (Santiago, Chile: Ministry of Health).
Note: The figures for infant mortality reflect the deaths of children ages 0 to 12 mo.

by respiratory and diarrheal disease in children less than age 1 yr (Table 1). A similar trend has been observed in preschool child mortality, which has declined from 14 per 1000 in 1960 to 0.6 per 1000 in 1994 and 0.4 per 1000 in 2002.

Meanwhile, the percentage of children with malnutrition has also been reduced dramatically, from 37% in 1960 to 2.9% in 2002. Second- and third-degree malnutrition similarly decreased from 5.9 to 0.2% (Table 2). Maternal nutrition has improved if we consider that the percentage of newborns with low birth weight (below 2.5 kg) has diminished from 11.6 to 4.8% from 1975 to 2000.

2. GENERAL CONSIDERATIONS

It is self-evident that malnutrition is the result of poverty and underdevelopment. Many authors have demonstrated that in any given country, there is a close relationship

Table 2
Percentage of Malnourished Children (Ages 0 to 6 Yr) in Chile From 1960 to 2000

Year	*Total*	*Mild*	*Moderate*	*Severe*
1960	37.0	31.1	4.1	1.8
1970	19.3	15.8	2.5	1.0
1980	11.5	10.0	1.4	0.2
1990	8.0	7.7	0.2	0.1
2000	2.9	2.6	0.2	0.1

Source: Informe Anual, 2000 (Santiago: Ministry of Health).

between the level of underdevelopment and the percentage of children suffering from malnutrition. In Chile, however, this correlation does not exist. The nutritional condition of children up to age 5 yr is much better than the socioeconomic realities of the country would predict (Fig. 2) *(2)*.

Similarly, studies have shown a close relationship between life expectancy and GNP per capita. Again, this relationship is not observed in the case of Chile, where the life expectancy is much higher than what would be expected from the GNP per capita (Fig. 3) *(3)*.

This anomaly calls for close analysis, because it demonstrates that under certain conditions, it is possible to improve health and nutrition even when there is no substantial economic development. These facts, as observed in Chile, contradict the claims of many economists who believe that progress in health and nutrition only can be achieved through a substantial degree of sustained economic development. From this view, economic growth creates new resources that permeate the different strata of the society, ultimately improving living conditions, even among the lowest socioeconomic groups.

From our viewpoint, however, this economic analysis is not plausible. Development itself cannot be achieved if a high percentage of the population is already damaged as a consequence of poverty and malnutrition and is barely able to survive because of poor health conditions. Malnutrition, poverty, and underdevelopment constitute a truly vicious cycle that works to hinder economic growth.

Considering these two different viewpoints and given an environment of limited resources, two different strategies arise for preventing malnutrition and improving health conditions: (a) focusing the effort on achieving large-scale economic growth and development; and (b) focusing efforts on targeted health, nutrition, and education interventions to improve the well-being of the population, especially the most deprived groups.

Regarding the first strategy, one might conclude that if a society were to devote all efforts to economic development and to generating expanded economic resources, then increases in wealth would benefit greater numbers of individuals over time, eventually reaching the entire population. However, two questions arise from this approach. First, how long would it take under the best circumstances for this wholesale improvement to occur, accounting for the realities existing in poor countries? Poor countries, for the most part, lack adequate technologies and the capability for either generating or acquiring these technologies. These countries tend to have inefficient infrastructures, lack trained workers, and have a low savings capacity and little investment capital. Also, they are burdened by a large foreign debt. Thus, the logical answer is that on average, economic development would take a long time, probably many generations, to accomplish. Second, is it is possible to have significant and continuous development in countries in which 30

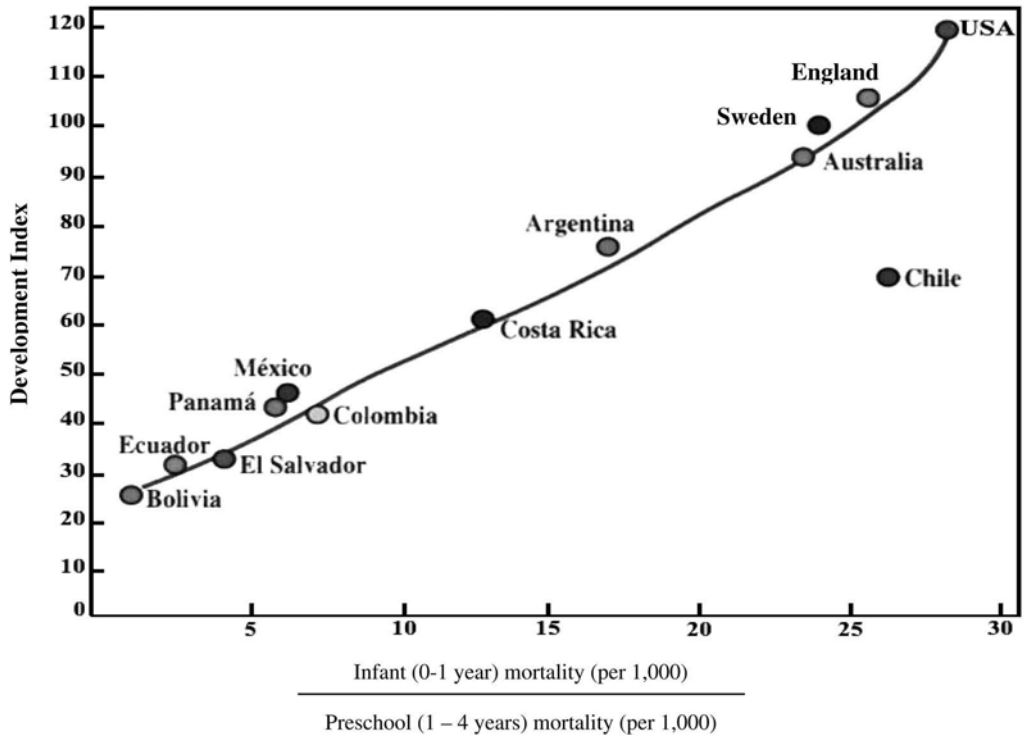

Fig. 2. Correlation between nutritional status of children below age 6 yr and degree of development in selected countries. Note: Degree of development has been calculated using an indicator developed by the Institute of Social Development of the United Nations, based on 80 different items. Lower numbers indicate less development. The infant and preschool mortality rates have been selected as indicators of nutritional status. (From ref. *2*.)

to 60% of the population has been physically and psychologically damaged over generations as a result of poverty and malnutrition? The answer, again, is negative.

If we seek to achieve socioeconomic development, which is the ultimate objective, the essential point is to break out of the vicious cycle created by underdevelopment, malnutrition, and poor health conditions. Human resources are one of the most important, albeit insufficiently recognized, factors for social development. Modern society has become very complex, and the demands on the knowledge and skills of its members grow constantly. Incorporating individuals as useful members of the community is unlikely if the environment deprives them of well-being and impedes full expression of their genetic potentialities.

Under these conditions, even if some economic improvement occurs, it is not likely to reach the lowest socioeconomic strata, where malnutrition and disease are most prevalent. This assertion is confirmed by many observations. For example, during the decade from 1970 to 1980, economic progress was observed in Latin America, but this only led to a widening gap in economic circumstances among different groups. Therefore, although the poorest 20% of the population did not increase its per capita income, the richest 10% increased its income by an avergae of about $400 *(4)*.

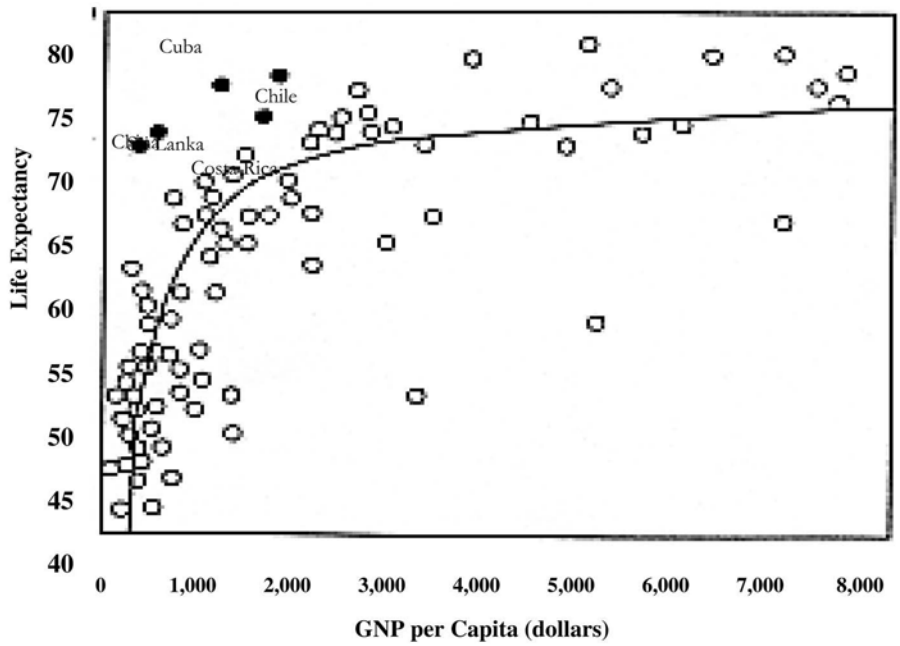

Fig. 3. Life expectancy in relation to GNP. Note: Line of central tendency is freehand curve. (Source: UNICEF Commission on Health Research Development, Health Research: Essential Link to Equity in Development. Oxford University Press, New York, 1990, p. 10.)

Protecting the human resources of a country is a difficult, long-term task. At least four areas must be considered: health, nutrition, sanitation, and education. A strategy has to be adopted to develop the human resources in each of these four areas and to coordinate programs among the sectors. Individuals must be protected from the moment they are born (or even before) by adequate nutrition, sanitation, health care, education, and housing. These components must be well-coordinated because they are interrelated and interdependent.

It is obvious that all nations would benefit from a nutrition and health policy. However, a universal health and nutrition policy would require considerable resources, and the ultimate decision rests at the political level. In a situation of underdevelopment, governments have many urgent priorities to confront, and often, political decisions on health and nutrition take a back seat and are postponed. Unfortunately, those who suffer from extreme poverty and malnutrition are not well-organized and lack the means to exert political pressure. Furthermore, results take a long time to become visible, and policymakers frequently need immediate results.

In my experience, political decisions supportive of a national health and nutrition policy do not happen spontaneously. Rather, they must be induced. In the case of Chile, the University (Universidad de Chile)—especially our Institute (Instituto de Nutrición y Tecnología de los Alimentos, INTA)—has played a very significant role. Pragmatically, whereas the first priority of a politician is to achieve power, the first priority of a government is to remain in power. On the basis of this assumption, support for a desired political decision is obtained only when the decision brings benefits to political

supporters, that is, to provoke a favorable political decision, malnutrition and health problems must become visible political issues.

For many years, we have developed a defined communications strategy to create awareness of nutritional and health problems in the community. With this purpose in mind, for years we have used the mass media, including the training of journalists, to create awareness about the adverse effects of malnutrition and poor health on individuals and the whole society.

Only when this communications stage has been reached will politicians become disposed to incorporate health and nutrition programs or interventions into their political platforms. Although their actions are sometimes employed for propaganda purposes, substantive policy and technical criteria have slowly gained ground in this way. Therefore, a stage is reached when practically all the candidates for public office have programs aimed at eradicating malnutrition and improving health conditions. In this sense, election periods are especially important.

Generally, to be successful as a planner, it is necessary to calculate not only the nutritional or the health cost–benefit relationship of a given program but also the political costs and benefits. Only those professionals who have calculated the nutritional cost–benefit ratio of a given program are be able to translate that data into a political equation, having proved that they are successful in getting their ideas implemented.

Another notable consideration is to implement nutritional and health policies that last over time, beyond the tenure of the current government. Very frequently in Latin America, new governments have a tendency to change what the previous governments have done. The success observed in Chile exists because, in great part, programs have been maintained and perfected over time. In this respect, our institute has played a crucial additional role *(5)*. Every political system or government has its supporters and enemies. Programs, with their successes and failures, necessarily come to be identified with the government in power. Therefore, professional planners responsible for these programs will also come to be identified with a particular government.

The crucial point for the professional is to reach an acceptable balance between political involvement and independence. This is perhaps the key point: to keep the programs running beyond the term of the government in power. In this sense, we have been in an exceptional position, because our interventions have been carried out in a university setting and from the vantage point of a highly respected institution. It is essential to gain the confidence of the government in power as well as of the community. The whole team of experts participating in a health and nutrition program must be cautious not to get involved with contingent, changing, and short-lived issues. This is extremely difficult, especially in Latin American universities, because they can easily become instruments to promote a particular political point of view, despite the essential need to remain outside of the power struggle. Therefore, it is important for professionals to win support from all sides and to remain aloof from partisan political battles.

Finally, a nutritional and health policy must be flexible in that it must be adaptable to the socioeconomic strategy of the government. Often, planners try to do exactly the opposite, that is, to adapt government socioeconomic policies to the needs of a nutrition and health policy. Lack of flexibility is unrealistic and underlies many failures. Policy must be developed in a way that it is compatible with different political philosophies and realities, ranging from a Socialist economic system to a liberal, market economy. This has been the case in Chile, where nutritional and health policies have survived shifts from one extreme

to the other. During the past 40 yr, despite changing governments and economic strategies, the basic principles behind health and nutrition have been maintained, and program efficiency and coverage have been improved. Additionally, new interventions have been implemented regardless of changing circumstances. This means that health and nutrition policies were flexible and were not linked to any particular political group or ideology.

3. PRIMARY HEALTH CARE

In a underdeveloped country, a high percentage of people are born into extremely impoverished conditions and are subject to high risk. They also are totally marginalized, living outside the socioeconomic structures of society. Therefore, it is difficult to reach them with the basic services provided by the government. For most families, this poverty has persisted for many generations. Furthermore, they do not anticipate any change in their circumstances, nor do they perceive the benefits that they could obtain from the services provided by the government. People living in extreme poverty frequently lack the capacity to become organized, and they do not and cannot exert political pressure to obtain favorable legislation. Many governments have organized health systems for groups that can exert pressure, such as blue- or white-collar workers, the military, and the middle class, but ignore or pay little heed to marginal rural and urban groups.

From our viewpoint, the first step in a sound nutrition and health policy is to organize a national health infrastructure. This would cover the entire population, especially the lower socioeconomic groups, and offer free services for those who cannot afford them. This policy has taken effect in Chile. In 1952, several organizations that provided health care were merged and transformed into the National Health Service. Since then, preventive medicine has been provided, at no charge, to the entire population.

In the beginning, only a small percentage of the population was covered; however, coverage has extended to the whole country, excluding only those who can afford private medical care. Presently, the National Health Service has 38,563 hospital beds and 1520 health clinics and health centers throughout the country. The National Health Service employs 58,000 persons, including 5300 physicians, 1602 dentists, 4002 registered nurses, 1690 midwives, 836 social workers, 790 nutritionists, and 30,200 nurses aides.

In the 30 yr that the service has been operating, coverage has been not only extended but has been made more effective. The personnel has developed an attitude of service and commitment to the community and have gained its respect. In turn, the population has become aware of its rights and responsibilities regarding health care. For example, examinations during pregnancy have increased considerably, 99.9% of births now take place in hospitals, and an estimated 97% of all children are administered immunizations regularly. As a consequence, the incidence of diseases such as whooping cough has declined considerably, and poliomyelitis, measles, and tetanus neonatorum have been eradicated. Regular check-ups of children have become routine and provide weight-for-age data for more than 96% of preschool children, who are evaluated every 3 mo.

Family planning has been included among health activities since 1966 and has resulted in a considerable decrease in fertility. Currently, a large percentage of women are using contraceptives, which has contributed to a decline in population growth from an annual rate of 2.9% to the present rate of 1.32%. The decrease has taken place mainly in the low socioeconomic groups. Because of this, a significant decrease has been observed in infant mortality and malnutrition. From 1962 to 1981, the crude birth rate

Table 3
Amounts of Food Distributed Monthly by National Health Service to Children and Mothers

			Composition per 100 g	
Group	*Product*	*Amount (kg/mo)*	*Calories*	*Protein (g)*
Children				
0–5 mo	Milk 26% fat	3	496	27
6–23mo	Milk 26% fat	2	496	27
2–5 yr	Weaning food	1.5	420	20
Women				
Pregnant	Milk 12% fat	2	410	31.6
Nursing	Milk 26% fat	3	496	27

(From ref. *2*.)
Note: In each instance, "milk" means powdered milk.

decreased from 38 to 23 per 1000, particularly among families with four or more children, in which the greatest incidences of malnutrition are usually found. There also have been fewer births to women age 35 yr or older, a situation that has contributed to the decline in neonatal mortality. The decrease in the birth rate is responsible for 20% of the reduction in the infant mortality rate *(6)*.

From the beginning, a program of nutritional intervention was implemented throughout the National Health Service. It includes free distribution of powdered milk for every child up to age 2 yr and weaning foods for children between ages 2 and 5 yr. The program also includes powdered milk distribution to lactating and pregnant mothers (Table 3).

The food is distributed through the primary health care centers and acts as a motivation for undertaking other health care activities. Food distribution has been extremely important not only from the nutritional point of view but also as a mechanism to attract mothers to the health care centers. To achieve these goals, the products distributed and their packaging and display have been of excellent quality. Special care has been taken to avoid the feeling that these programs are designed for poor people. The same products and services compete successfully in the open market.

The food distribution program began in 1951. In the beginning, the amount of milk that was dispensed was limited but increased gradually as the National Health Service developed. Figures 4 and 5 show that a close relationship exists between the amount of food distributed and the number of visits to a primary health care facility. The linkage between food distribution and attendance at health care centers means that in the public mind, both programs are interrelated and complementary.

In short, an efficient health care infrastructure and rational nutritional programs have been two basic factors that explain the dramatic decline in malnutrition and infant and preschool mortality in Chile between 1960 and 1974. The improvements during this period served as a foundation for later progress. The implementation of the health system, with its broad coverage, has led not only to effective nutritional programs that are oriented at target groups but also to other interventions, such as family planning, health controls, immunizations, health and nutrition education, and the promotion of breastfeeding. The fact that the community has developed a true "culture of health" and has become aware of the need to preserve this culture through the health service is of paramount importance.

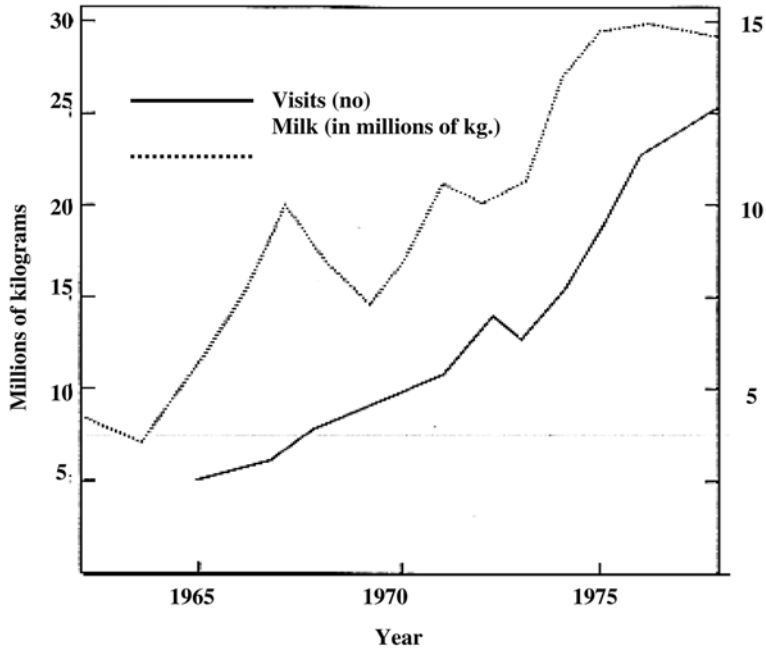

Fig. 4. Prenatal visit and amount of food distributed. Note: As food distribution increases, so does the number of visits of pregnant women. (From ref. *12*.)

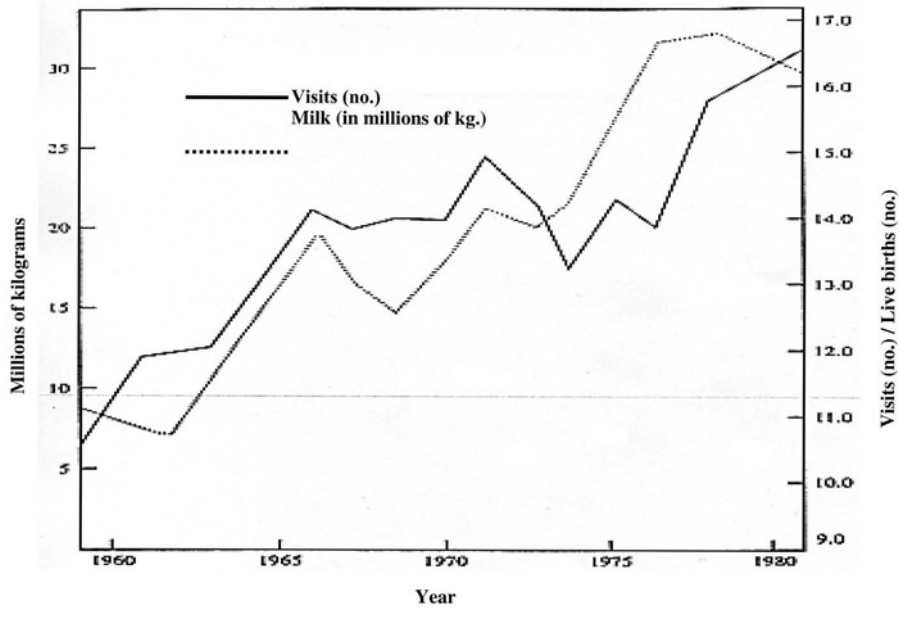

Fig. 5. Children attended by the National Health Service and amount of milk distributed. Note: As milk distribution increases, utilization of medical services. (From ref. *12*.)

4. OUR EXPERIENCE IN NUTRITIONAL POLICY

Despite all of these advances, in 1974, malnutrition in Chile still affected 19% of the preschool children, and infant and preschool mortality was 64 per 1000. Therefore, a new strategy was required, and for this purpose, the government created the Council for Food and Nutrition (Consejo Nacional para la Alimentación y Nutrición; CONPAN) in 1974. This was an autonomous, interministerial agency entrusted with the preparation and coordination of a nutritional policy for the country. The agency had a council that was composed of the ministers of health, economy, education, agriculture, work, social welfare, and planning. CONPAN had an executive coordinator to supervise the technical and administrative coordination of the agency's activities, a position that I had the privilege of undertaking.

CONPAN resulted in a new nutrition policy with the aim of ensuring the best possible nutritional situation for the entire population within the limits of the country's resources. Special emphasis was placed on protecting the most vulnerable groups. The new policy encompassed the entire food chain as well as the concerns for food security, food production, imports, food marketing, income, quality control, nutrition education, micronutrient disorders, nutritional diseases, and sanitation *(7)*.

We soon realized that these concepts were absolutely theoretical. To articulate a nutrition policy is one thing, to try to implement it is a completely different issue. Numerous obstacles became apparent. Resources were limited; bureaucratic resistance surfaced; vested interests emerged; and, finally, rivalries among different ministers arose, reflecting the fact that few would accept interference in their own areas of responsibility. We learned the hard way that an ideal nutrition policy may be an impossible dream and that it may be unnecessary after all. Therefore, we concentrated our efforts on specific interventions aimed at improving the nutritional condition of specific target groups.

CONPAN had a short life and was disbanded by a government decree. Thereafter, we changed the strategy to establish direct relationships with particular ministers and key people in the different sectors. This approach proved to be much more effective. Our conclusion was that we should not pretend to have a comprehensive nutrition policy or an official body to coordinate the interventions of the different sectors. Coordination and evaluation are extremely difficult tasks in any government. The essential points are to exert leadership; to have credibility based on scientific and technical authority; and to exhibit flexibility, and—most of all—persistence and patience. Again, it should be noted that this has been the critical role played by the university and, more specifically, by INTA.

In retrospect, I think the rather brief existence of CONPAN was necessary. Methodologies for solving significant problems were developed, and many of the interventions that were designed have since been successfully implemented. Above all, the government became aware of the need for scientifically based programs that seek to help groups that have been excluded from the economic and political structures of the country. After describing this experience, I must say that ultimately, considerable progress was achieved during this recent period, which I attempt to summarize here by sectors—health and nutrition, education, and agriculture.

4.1. Health and Nutrition Sector

A data collection system was developed that was specifically targeted to the nutritional condition of children up to age 6 yr. Every child under the care of the National

Heath Service (an estimated 1.35 million children) is measured every 3 mo and his or her weight-for-age is calculated. Basic family information is gathered to examine socioeconomic conditions and related health risk factors. Information about weight at birth is regularly collected for all the deliveries in the hospitals of the country.

4.1.1. Food and Nutrition Interventions

Targeted food interventions have been developed for families in extreme poverty. For these groups, a food distribution program that includes staples such as rice, wheat flour, and oil was established through the primary health care centers. The objective of this program is to improve the nutrition of both the children and their families. This program selects families with low-birth-weight children (2.5 kg), children whose weight increase has been inadequate for 2 mo, and children born to mothers under age 20 yr. Families with five or more children and those with severe socioeconomic problems also participate in the program.

4.1.2. Treatment of Malnourished Children

Children suffering from severe malnutrition now have access to treatment. This program has had a direct influence on infant mortality. A study performed in 1974 showed that an average of approx 8200 children per year became severely malnourished. A follow-up study confirmed that infants with severe malnutrition prior to age 6 mo had an 85% chance of dying before they were age 1 yr. Analysis of cases of severe malnutrition showed that almost all occurred in children under age 2 yr, and 80% of the affected children were age 6 mo.

In 1975, a pilot project was started to tackle this program, with the aim of rehabilitating infants with severe malnutrition. For this purpose, a specialized Nutritional Recovery Center was set up with 40 beds for infants. Each malnourished infant remained an inpatient until he or she fully recovered; he or she was given appropriate food and underwent a program of cognitive, affective, and motor stimulation. The mother was involved in the treatment and received education and training. The results of this pilot study were impressive. Although inpatient mortality previously had been about 25%, no deaths occurred among the children in treatment, and very satisfactory rehabilitation was attained in both nutritional and psychomotor status (Table 4 and Fig. 6).

In view of the successes of the pilot project, INTA set up a private foundation known as the Chilean Nutrition Foundation (Corporación para la Nutrición Infantil; CONIN) to extend the pilot program throughout the country. Within 3 yr, with funds provided by the community (amounting to $9 million), 33 centers were built, equipped, and put into operation in different cities in the country. The installed capacity covers all needs. With 1500 beds, it is possible to treat 34,000 severely malnourished children annually, thus bringing them to full recovery.

Each center has 40 to 60 beds, full medical facilities, a kitchen with milk distribution, and a laundry. The staff encompasses a full-time manager and six health professionals, including a pediatrician, nutritionist, registered nurse, nursery teacher, and a social worker. Forty nurses aides work in three shifts, and 80 volunteers work on individual stimulation programs and follow-up visits at home. Operational costs are financed under an agreement with the National Health Service (which covers 80% of the costs) and by CONIN-raised funds *(8)*. The actual cost per day is $13.00 per child, and the total cost of rehabilitating one severely malnourished infant is $1170. CONIN has a staff of 1400 in addition to 2300 voluntary workers.

Table 4
Clinical Progress of Marasmic Infants During 4 Mo of Treatment

Statistic	*At admission*	*At 50 d*	*At 100 d*	*At 150 d*
Weight deficit for age (g)				
Group A	56 ± 8	54 ± 13	48 ± 12	40 ± 13
Group B	55 ± 5	35 ± 8	21 ± 6	16 ± 3
Height deficit for age (cm)				
Group A	76 ± 10	70 ± 17	65 ± 14	65 ± 14
Group B	82 ± 13	50 ± 14	32 ± 6	21 ± 7
		$p < 0.001$	$p < 0.001$	$p < 0.001$
Psychomotor development quotient				
Group A	56 ± 8	60 ± 11	64 ± 14	65 ± 12
Group B	55 ± 5	71 ± 10	80 ± 8	85 ± 1
		$p < 0.001$	$p < 0.001$	$p < 0.001$

Note that group A consisted of 80 infants treated in a conventional pediatric hospital and group B consisted of 80 infants treated in a CONIN center.

(From: Mönckeberg F. Nutrition in the 1980s. Liss, 1980, p. 141.)

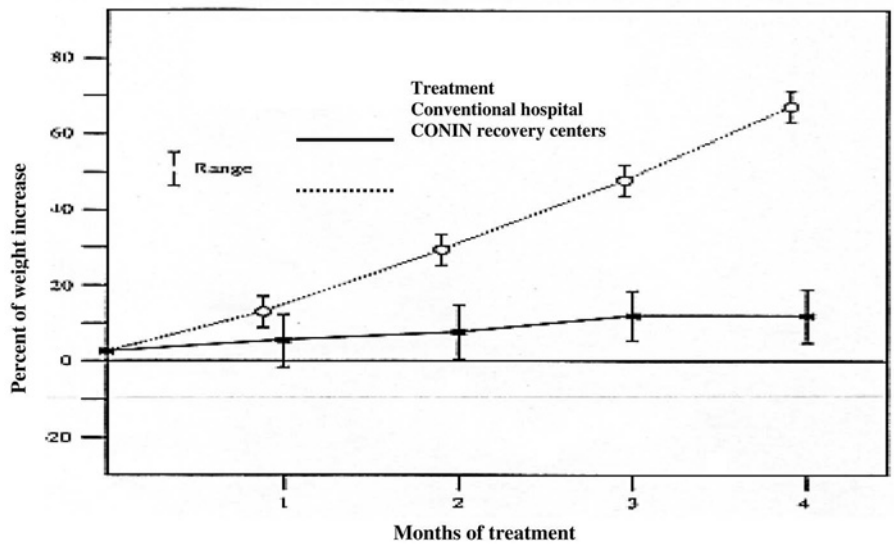

Fig. 6. Weight increase in 70 severely malnourished infants after 4 mo of treatment. (From ref. *8*.)

The program has had a notable impact in reducing the number of children suffering from severe malnutrition and in reducing the incidence of infant malnutrition. It is estimated that CONIN helps prevent almost 2000 deaths annually among infants under age 12 mo. Thus, in terms of overall infant mortality the program is responsible for over 20% of the reduction in infant mortality. During the years in which the program has been operational (1975–2002), more than 85,000 infants have been rehabilitated, with an average mortality rate of 2%. Follow-up studies show that 98% of the children who have recovered continue to have satisfactory weight gain at home, and only 1.8% have required re-admission in a center.

CONIN now has embarked on a new program that rehabilitates low-birth-weight babies before returning them to their mothers. Studies have shown that this is a high-risk group that significantly contributes to mortality during the first 2 mo of life. Affected infants remain in a center for 30 d, during which time the mothers receive instruction on child care *(9)*.

4.1.3. Breastfeeding

Because a large share (85%) of Chile's population lives in urban areas, and perhaps as a result of the system of free milk distribution, there has been an abrupt decline in the practice of breastfeeding. In 1940, 85% of the infants were breast-fed up to age 6 mo. By 1967, this proportion had decreased to 25%, and by 1974, the proportion was only 19% *(10)*. This decline undoubtedly contributed to the increase in early, severe malnutrition. Consequently, an extensive program was introduced to promote breastfeeding, using both the mass media (e.g., radio, television, magazines) and formal education. In particular, the professional medical community and the schools that train child health care workers (such as midwives, nurses, and health educators) took a firm stand regarding this issue. Professional and auxiliary personnel have received instruction provided by the National Health Service. Printed materials have been prepared and distributed that illustrate the importance of breastfeeding and how to advance its occurrence.

As a consequence, there has been a noticeable change of attitude in the population and among health care personnel. Breastfeeding has significantly increased. More than 70% of mothers now breastfeed their children during the first 90 d of life, representing an 88% increase in breastfeeding during the first 3 mo of life *(11)*.

4.1.4. Nutrition Education

A nutrition education program was created by CONPAN and included nutrition training at both the elementary and intermediate school levels. Textbooks have been prepared, although they have not been used. However, in the health sector, there has been considerable progress with nutrition training. It has been possible to staff each national health service clinic with a nutritionist, who is responsible for implementing the nutrition education program. Nutritionists, who are required to undergo 5 yr of university courses, have carried out important work in both the prevention and the treatment of malnutrition.

4.1.5. Rural Health Care

Because 85% of Chile's population is urban, the National Health Service has adequate coverage in urban areas; however, it has been more difficult to provide coverage in rural areas, especially in the most isolated regions. Medical and nutritional care for rural communities is provided by health posts, each of which has a full-time health worker whose activities are complemented by regular visits from health teams. These health posts also provide nutrition programs.

To increase the availability of health care for pregnant rural women and to provide adequate access to the nearest maternity facility, a program known as Hostels for Rural Mothers was implemented. The idea was to build these accommodations near maternity hospitals and to allow pregnant women from rural areas to stay there during the 15 d prior to and up to 10 d after the delivery. The women also receive instruction from hospital staff concerning nutrition, breastfeeding, and child care. Sixty-four of these hostels

have been established in conjunction with maternity hospitals in primary health care areas. Together with other measures, they have significantly decreased neonatal mortality by reducing the risks inherent in delivery.

4.2. Educational Sector

4.2.1. Primary Education

During the past 30 yr, a considerable improvement has occurred in elementary education. In 1960, more than 30% of the population was illiterate; the illiteracy rate now stands at less than 2%. Only 10% of the children in 1960 had completed elementary education. Today, 100% of children complete 8 yr of elementary education.

It is particularly important to draw attention to the emphasis that has been placed on education for mothers, because this has a strong bearing on malnutrition and infant mortality. The latter is five times higher among mothers who have not completed elementary education than among those who have *(12*). Progress in the educational field is particularly noticeable among women who were illiterate during their childbearing years. This figure fell to 6% by 1982 and 1% in 2002. In the period from 1969 to 1982, there was a noticeable decrease in the number of births to women with a low level of education (i.e., either without education or with only elementary level education).

4.2.2. School Food Programs

The school feeding program, although not technically a nutritional intervention program because it only can provide limited daily calories and protein, has had a very important effect in reducing school desertion. CONPAN reviewed the program and reached the conclusion that its implementation should be transferred to private agencies that could provide the complete service and left the role of evaluation and control to the Ministry of Education. This re-organization improved the quality of the service and accomplished the goal of combating school desertion. Presently, the program, which takes place during 180 d of the school year, supplies 1,350,000 breakfasts and 830,000 lunches daily to 5000 schools across the country.

4.2.3. Preschool Food Programs

The largest proportion of malnourished children in Chile live in deprived urban areas as a result of the massive migration to the cities, which took place during the past decade. Almost every city has a belt of shantytowns surrounding it. Experience shows that it is difficult to prevent malnutrition in children from population groups with a low socioeconomic and educational level, even when free food is provided. Moreover, several studies indicate that extreme poverty causes damage to the physical and intellectual capabilities of individuals. This damage is the result not only of malnutrition but of other factors inherent in chronic poverty.

Against this background, CONPAN supported the development of a day care center program for preschool children living in extreme urban poverty. Children attending the centers during the day have all food provided and also undergo psychomotor and affective stimulation. During the past 15 yr, 1500 such centers have been built and equipped, covering 360,000 preschool children between ages 2 and 6 yr. According to earlier estimates by CONPAN, the program will eventually cover 380,000 children, which is the number believed to be at risk of malnutrition and living in extreme poverty in urban areas *(13)*.

4.2.4. Homeless Children

A program for homeless and troubled children was designed to provide care in live-in centers for children ages 2 to 12 yr. These children include orphans, abandoned children, and children who display maladjusted behavior. The goal is to restore the child to a normal behavioral situation, to combat child vagrancy, and to improve the conditions of life. The program has been extended and improved through the building of new centers and now provides coverage to 52,000 children.

4.3. Sanitation Sector

Overcrowding and inadequate sanitation in urban areas are common to all Latin American countries. The situation has become worse in the past few decades as a result of the explosive growth of the population and increased migration to the cities. In 1974, a high percentage of the population in Chile lived in extremely impoverished areas that lacked sanitation facilities. Inadequate sanitation adversely affects child nutrition and gives rise to gastrointestinal and other infectious diseases. Diluted, contaminated infant formulas are one of the main contributors to a shift in the incidence of malnutrition to the early months of life, and this, in turn, is associated with high risk of death.

For these reasons, CONPAN developed a program that emphasized the importance of sanitation in preventing early malnutrition. During the initial stage of this program, a brick-and-timber sanitary unit was built on the plots of 300 families that lived in a slum area. Each unit had a kitchen, a bathroom, and an outdoor sink for washing clothes. The kitchen was equipped with shelves and a sink; the bathroom had a lavatory, a flush toilet, and a shower. Hot water was also supplied. The unit was connected to the general sewage system *(14)*.

The results demonstrated a significant causal link between environmental sanitation and improvements in both nutrition and the quality of life. The most significant result may have been the striking change that occurred in the attitudes of the families. The pessimistic and fatalistic approach to life, the hopelessness, and the acceptance of the miserable lifestyle that prevailed in the community that existed before the sanitary unit was installed changed into a sincere and deeply felt desire for improvement in living conditions. This resulted in increased individual efforts and in motivation to meet the challenge of improving the quality of life.

The results led to the development of a nationwide sanitation program to improve the sanitary situation of the urban population. In the past 15 yr, 250,000 sanitary units have been built in different cities of the country. In 1960, only 40% of the population had drinking water in their homes, and only 35% had an adequate sewage system. At present, 99% of the population have drinking water at home, and 93% live in housing connected to a sewage system (Table 5). There is no doubt that improved sanitary conditions also have been important factors in the decrease in malnutrition and in the improvements in health conditions.

4.4. Agricultural Sector

Agriculture and food production are fundamental elements in any nation's development process and a precondition to raising the state of health and nutrition among people. As I emphasized earlier, the economic development of Chile (as expressed in GNP per capita) for the most part has not been very considerable during the past 40 yr. From the beginning of the 21st century, the Chilean economy has been primarily based on the

Table 5
Urban Coverage of Potable Water and Sewage Services (1970–2000)

Year	*Potable water (%)*	*Sewage (%)*
1970	72.3	41.2
1980	92.5	65.3
1990	97.3	78.1
1994	98.9	88.7
2000	99.2	93

export of raw materials—first nitrates and later copper. Industrial development began only after World War II, but this development was based on import substitution, with a high degree of protectionism. Agricultural development was retarded because of artificially fixed prices, the high cost of imported commodities, and the lack of incentives. As a consequence, agricultural production increased less than population growth, and the failure of agricultural production to keep pace resulted in the ever-increasing importation of food.

In 1970, a Socialist government was elected, and an agrarian reform was implemented. This reform resulted in the expropriation of more than 60% of the total land under cultivation in the country. Agricultural production fell almost totally under government control. A year later, agricultural production had nearly collapsed. Enormous amounts of food (equivalent to 70% of all the requirements) had to be imported *(14)*.

Three years later, in 1973, a military defeated the Socialist government, and a free and open market economy was created. In 1974, CONPAN and the Ministry of Agriculture established a new agricultural policy based on the following principles:

1. Transfer of state-owned land to peasants and farmers.
2. Free market prices for every agricultural product and elimination of all state subsidies.
3. Guaranteed prices for basic products.
4. Technology transfer to farmers.
5. Construction of an irrigation infrastructure.
6. A new credit policy.

As a result of these changes, a rapid increase in agricultural production was achieved, with an annual growth rate of 7% during the past 10 yr. The value of annual food imports fell from 700 million in 1973 to 195 million at present. In the past 11 yr, a remarkably strong agricultural sector has been established that consists of fruit and vegetable production and agroindustrial development. Currently, this has become one of the most dynamic economic sectors, which in 1995 accounted for $400 million in exports per year. The success of Chile's agricultural sector has led not only to a sharp decline in costly food imports but also to a substantial increase in rural employment and income and, correspondingly, to a marked improvement in health and nutrition.

5. FINAL COMMENTS AND RECOMMENDATIONS

The Chilean experience demonstrates that it is possible to implement targeted interventions in health, sanitation, education, and food production and distribution, which will result in substantial improvements in the health and nutrition status of the population despite the persistence of poverty and underdevelopment. This concept is important

because it shows that in Chile, it was possible to create conditions that prevented the negative physical and psychological consequences of an unfavorable situation that affected a large number of infants and children. By hindering the full expression of the genetic potential of the population, the prevalent negative societal and environmental factors impeded development. We maintain that the potential and capabilities of the population constitute the most precious asset of any country. To attain satisfactory levels of development, these resources must be carefully protected and nurtured. Of course, the successful application of our experience in other countries requires a careful analysis of the local realities and the adaptation of the programs to these realities.

In the early 1960s, Chile had one of the highest infant mortality rates in Latin America (120/1000) as well as the highest rate of malnutrition among children under age 5 yr (46%). Since then, a progressive, steady improvement occurred in the health and nutrition of infants and preschool children and this has continued to today. Biomedical indicators show that Chile currently enjoys some of the best indices in the region. The largest proportion of these improvements was achieved at a time (1970–1987) during which the GNP "per capita" did not change substantially and during which poverty persisted almost unchanged. Later, from 1986 to 2000, the situation in Chile began to improve from the economical viewpoint (as a consequence of a shift in the economic model) from a planned, centralized economy to an open, free market-oriented, liberal economy. As a result, during the past 15 yr, the GNP has increased at rates between 7 and 9% per year (*15*). During the same period, the percentage of population living in poverty has decreased from 40.7 to 16.2%. The infant mortality rate is now close to 9 per 1000, and the percentage of malnourished children under age 6 yr decreased from 8 to 2.9% (Table 2).

Currently, 100% children finish 8 yr of basic education, and 83% finish high school. There is no doubt that this socioeconomic progress is explained by a number of factors; however, it is also possible to postulatethat the change and improvement in the quality of the human resource has played a very important role during this process (although this may be difficult to prove). As an additional parameter of this change, it is worth noting that the average 18-yr-old Chilean male is 11 centimeters taller than his homolog of 30 yr ago. In other words, a higher percentage of children currently are expressing their full potential for growth.

The Chilean experience demonstrates that it is possible to prevent the damage caused to the population by this adverse environment, although underdevelopment and poverty persist. At a later stage, if the quality of the human capital is preserved and has a satisfactory level of education, it is possible to achieve substantial changes in the standard of living through sustained economic and social development.

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31 Effect of Westernization of Nutritional Habits on Obesity Prevalence in Latin America

Analysis and Recommendations

Jaime Rozowski, Oscar Castillo, and Manuel Moreno

KEY POINTS

- Nutritional habits of Latin Americans show a trend toward a Western-type diet.
- There is a clear tendency of increased consumption of fats, saturated fats, and animal proteins and of decreased consumption of fruits and vegetables in the diet.
- Prevalence of obesity is high in many countries of the region in all age groups, with a clear tendency to increase.
- Prevention programs should be implemented from childhood, with a strong political backing and the involvement of the community.
- Recommendations on objectives and goals to increase nutrition education and promote exercise in the population are proposed.

1. INTRODUCTION

Latin American countries, although located in the same region, show a substantial heterogeneity in their ethnicity, socioeconomic level, and health. The region (including the Caribbean) is home to 542 million inhabitants and is the most urban region in the developing world *(1)*. The region shows great inequalities in socioeconomic level, with more than one-third of the population living in poverty. For these reasons, it is extremely hard to discuss average values of any indicator used. For instance, although the average infant mortality rate in the region is 19.7 per 1000 live births, the range observed in the different countries is between 3.7 and 129.3 per 1000 live births. Similarly, life expectancy at birth, although showing an average of 70 yr, ranges from 56.8 to 79 yr, a 22-yr difference *(1)*.

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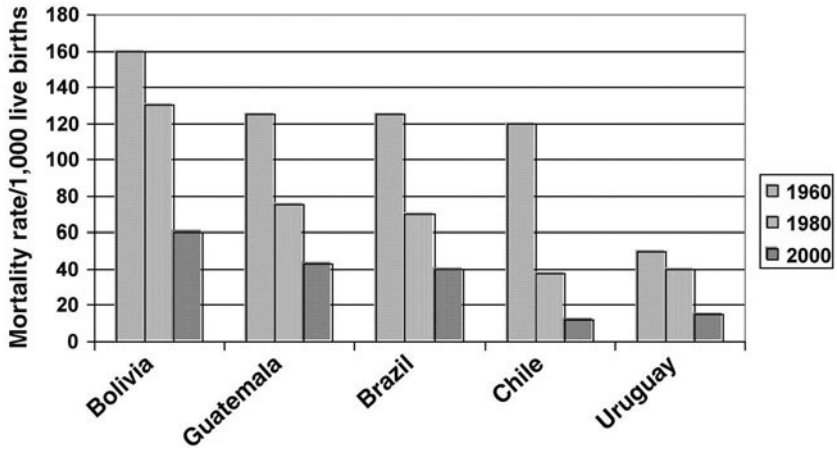

Fig. 1. Infant mortality rates in selected Latin American countries, 1960–2000. (From Pan American Health Organization *[1]*.)

Urbanization has been a major issue in Latin America during recent decades, involving currently more than 70% of the population. Of this 70%, almost 80% of them live in cities with more than 700,000 inhabitants. Although urbanization in industrialized countries usually brings along progress in technology and social development, in developing countries, the benefits of urban growth tend to be present only in the higher socioeconomic levels. In the low-income groups, urbanization usually leads to a relative decrease in income, which, from the nutritional point of view, produces a shift toward consumption of high-calorie foods with a reduced nutrient density, producing a trend to incorporate food into the diet that is more commonly consumed in industrialized nations. In fact, this trend is observed in the majority of the countries in the Latin American region in what could be called the "Westernization of eating habits" (*see* Section 3.); this trend is characterized by an increased consumption of the so-called "fast foods" coupled with a tendency to decrease physical activity.

The incorporation of these new trends has led the Latin American region as a whole and every country in particular to change morbidity and mortality profiles, producing an increase in the prevalence of chronic diseases greater than that of infectious diseases, the common killers of the past. Currently, chronic diseases are the main cause of death in most Latin American countries *(1)*. An example is the case of Chile, where the infant mortality rate in 1955 was approx 120 per 1000 live births, mainly as a result of malnutrition and infectious diseases. At that time, cancer and cardiovascular diseases (CVDs) accounted for 25.8% of all deaths in the country *(2)*. Currently, the infant mortality rate is around 10 per 1000 live births, with cancer and CVDs accounting for more than 50% of all deaths. Additionally, the mortality rates attributed to diarrhea and gastroenteritis of presumed infectious origin decreased from 20 to 0.035 per 1000 live births in the same period of time *(2)*. A similar pattern has been observed in other countries of the region. Figure 1 shows the infant mortality rates from 1960 to 2000 in five countries representing a range of socioeconomic levels. Although all of these countries show a reduction in infant mortality rates, this decline has been variable, with Chile demonstrating the largest relative decrease in the last four decades. These data are also reflected in the

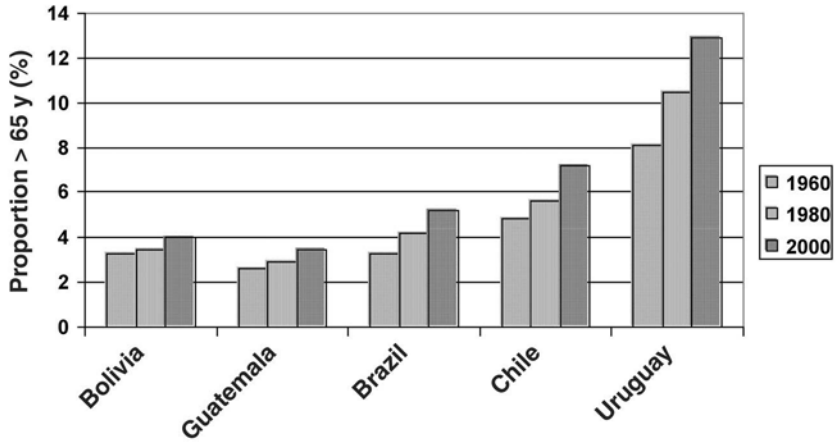

Fig. 2. Proportion of people older than age 65 yr in selected Latin American countries. (From the Economic Commission for Latin America *[3]*.)

proportion of individuals older than age 65 yr, as shown in Fig. 2, which also depicts data since 1960. The proportion of the group older than age 65 yr has increased 66% in Brazil and 50% in Chile and Uruguay since the 1960 *(3)*. In countries with slower development, such as Bolivia and Guatemala, the increase in this segment of the population has been minimal. Aging of the population, coupled with a decline in early deaths, forecasts an increase in the prevalence of the chronic diseases of the aged.

The increase in adult population causes a growing demand on health services, not only to take care of the nutritional needs of the mother and child (undernutrition and micronutrient deficiency) but also to provide for the treatment of nontransmissible chronic diseases and their associated risk factors. Therefore, it is very important to decrease the nutrition-related risk factors involved in these diseases, such as excessive weight and high intake of saturated fats, through health promotion and nutrition education in the community.

2. SOCIOECONOMIC CHANGES

A thorough discussion of socioeconomic changes in the Latin American region is beyond the scope of this chapter. However, it is important to discuss some aspects that are relevant from the nutritional point of view. The gross national product (GNP) of the countries in Latin America has increased substantially in recent decades. Nevertheless, this increase has not always been accompanied by an improvement in the health situation. For instance, Brazil, which in 1990 had one of the largest GNPs per capita in Latin America, still had an infant mortality rate of 57.5 per 1000 live births, higher than the rates of countries with lower GNPs, such as Chile and Uruguay *(4)*.

The improvement in GNP and in the general well-being of many countries in the region has produced an influx of foreign fast food enterprises that offer a wide range of food items at very low cost and, therefore, at the reach of people from all socioeconomic levels. For instance, a chain of fast food restaurants had a recent offer in Santiago, Chile in which hamburgers were advertised for the equivalent of $0.60. At this price, practically everyone was able to take advantage of this offer.

Another important factor has been the increase in portion sizes, which have become progressively larger in recent decades. Recently, Rolls et al. *(5)* showed that larger food portions led to greater energy intake in volunteers, independently of body mass index (BMI).

The problem becomes more complex upon examination of the availability of nutritionally balanced foods. For example, in the case of Costa Rica, the proportion of income necessary to purchase the basic food basket in 1987–1988 was such that 20% of the population needed far more than the average income to purchase the basket *(6)*. Another 40% of the population needed between 40 and 60% of their income for the same purchase, indicating that for a large proportion of the population, it takes a major effort to properly feed themselves. It is obvious that this induces people to consume cheaper foods that usually are low in nutrient density and high in calories.

3. FOOD HABITS

A thorough analysis of food habits in Latin America is hampered by the lack of national food-consumption surveys. With the exception of a few countries, most information available is from small surveys that often are not representative of the population. Additionally, most of these surveys are published in local journals that are not always widely available.

There is a great diversity of national nutritional habits in Latin America. In the Southern Cone countries (Chile, Argentina, and Uruguay), wheat represents the main component of the national diets, with the addition of meat and dairy products in Argentina and Uruguay. Corn is the main staple in Mexico and Central America (with the exception of Costa Rica). The rest of the countries in that area have a rather diverse diet, combining the three main cereals (wheat, corn, and rice). In the Andean countries (Peru, Bolivia, Ecuador) and Paraguay, potatoes represent an important component of the diets. In many countries, sugar has become an important contributor of calories, representing about 10% of caloric intake on average. However, in some groups, it can provide as much as 20% of dietary calories *(7)*.

Socioeconomic level is a major determinant of food intake. The structure of national diets has shown a tendency to change in most of the countries when income per capita has increased *(8)*. Availability of lipid calories has increased because of a higher supply of fat (butter, margarine, and different types of oils), with a high proportion of this coming from animal fat. Calories from complex carbohydrates have shown a tendency to decrease in time, whereas the calories from sugar and other caloric sweeteners tend to increase, as has been shown worldwide across different socioeconomic levels *(9)*.

Protein calories tend to remain stable or to increase slowly but show a rapid rise in the intake of proteins from animal origin. One example of this is demonstrated in Fig. 3, which shows changes in consumption in Brazil. The decrease in intake of fruits and the increase in the consumption of dairy products and vegetable oil is remarkable. Generally, the higher income group consumes a diet richer in saturated fats when compared with the groups with lesser income *(10)*. Additionally, increases in BMI parallel increases in income and dietary fat content *(11)*.

In Chile, the last national nutrition survey in 1974 showed that although only 5% of the population in the highest income quintile was consuming less than the requirement of protein (0.75 g/kg of body weight), 51% of the poorest quintile consumed less protein than the requirement *(12)*.

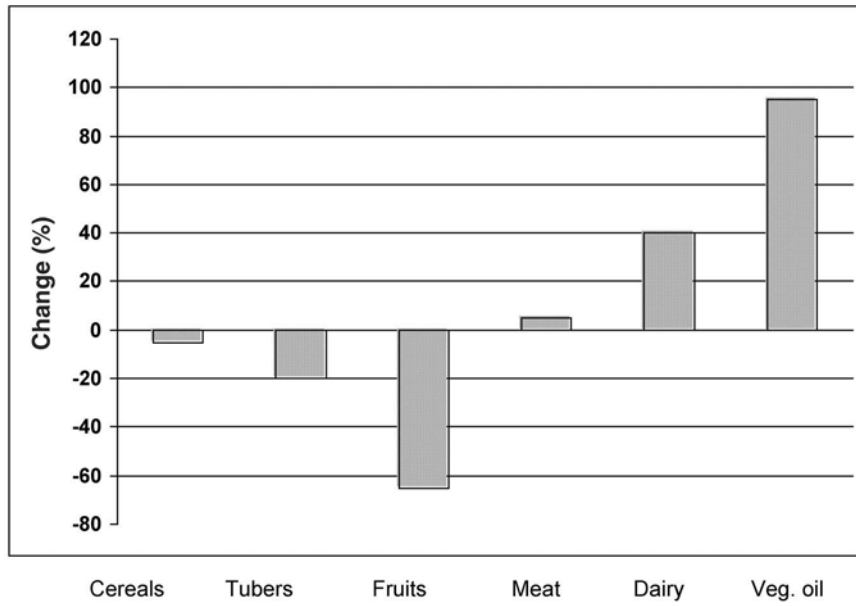

Fig. 3. Average changes in food consumption in Brazil, 1962–1988. (Adapted from ref. *10*.)

Generally, family income and urbanism are important determinants of the differences in food regimens among families within the same country. Urban families from low socioeconomic levels have an intake that is about 40% greater in cereal calories than that of high socioeconomic level families. People in the upper socioeconomic level have an intake of animal proteins that is 80% greater than the poorer levels *(7)*. As average income increases, countries tend to adopt a food model that is characterized by a high intake of energy, animal proteins, and fats, with a tendency to replace simple and traditional foods by industrialized and widely distributed products *(13)*.

Here, we compare the availability of different nutrients in countries of Latin America. We have chosen to use availability of food provided by the Food and Agricultural Organization rather than compare nutrition surveys, which are often based on different methods (24-h recall, food registry, etc.), usually done in small populations, and may not always be reliable *(14)*.

3.1. Energy

There is great variation in the amount of calories available in the different countries from the Latin American region. Most countries have shown an increase in availability of calories—particularly in the case of Peru and Ecuador, which showed an increase of almost 400 calories between 1981 and 2001 (Fig. 4). In other countries, availability of calories has slightly decreased or showed no significant change.

3.2. Fats

There is a clear tendency in the region to increase the availability of fats, with 14 countries showing an increase over 20 yr (Fig. 5). Nevertheless, only two countries, Argentine and Uruguay, display an average proportion of calories from fats that could be considered

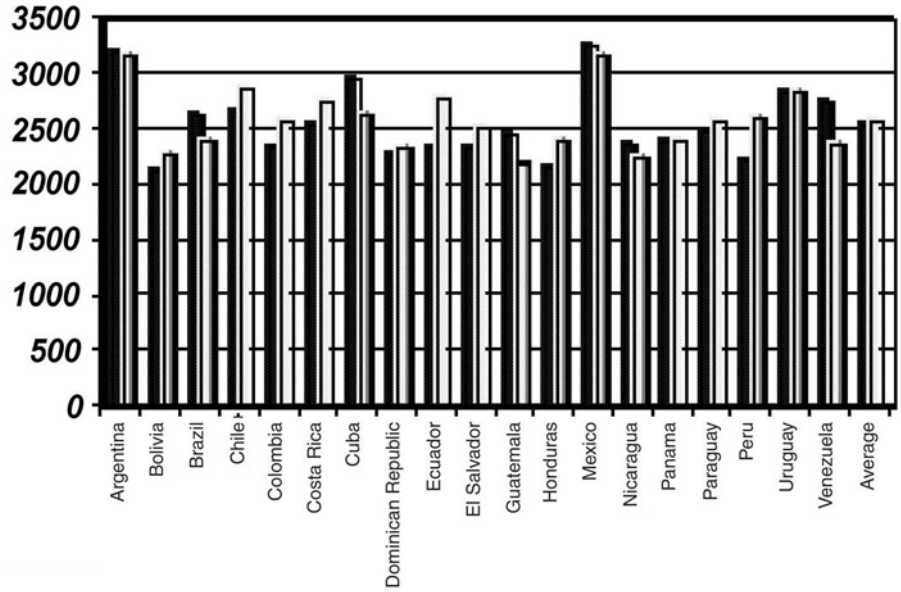

Fig. 4. Changes in availability of energy in Latin American countries, 1981 and 2001. (From FAO *[14]*.)

elevated, although their availability has not increased. The increase in fat consumption is a hallmark of the Westernization of the diet. This has been confirmed in several countries *(11,15,16)*. This particular pattern of fat consumption has been shown to correlate with an increase in blood lipids *(17)*. Regarding the type of fats consumed, there has been a shift from fats of vegetable origin to animal fats, probably because of the increased consumption of meat and prepared foods, which consequently has led to an increased intake of saturated fats.

3.3. Carbohydrates

Generally, most countries from the region show a decrease in carbohydrate consumption, with the exception of Cuba. More importantly, they also show a shift from complex to refined carbohydrates (mainly sugar). This results partly from a change from traditional dishes to the more readily available prepared foods that usually are ready-to-eat.

3.4. Proteins

Protein availability in the region has remained constant over the past 20 yr, with a tendency to consume relatively more protein of animal origin, which results in an increased intake of saturated fats (data not shown).

The changes in food availability are the result of a transition from consumption of traditional dishes (usually based mainly on cereals) to a more convenient and faster pattern of eating that tends to increase the consumption of calorie-rich products, fats, and salt. As mentioned earlier, cereals historically have been the main staple in Latin America, followed by vegetables, fruits, and roots. However, this pattern is changing to a diet that is more refined and has more sugar, fats, animal proteins, salt and less fruits, vegetables, and roots.

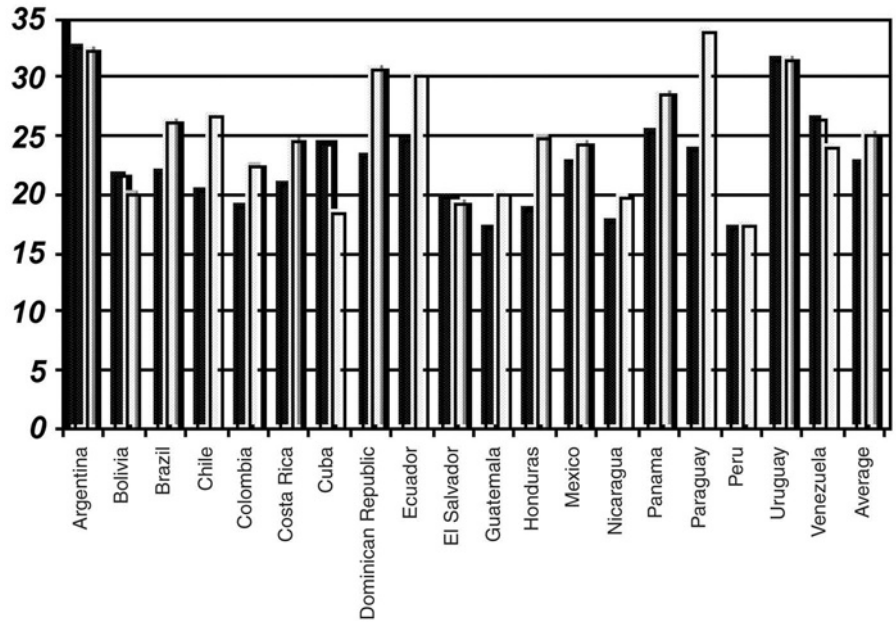

Fig. 5. Changes in fats availability in Latin American countries, 1981 and 2001. (From FAO *[14]*.)

A notable case occurred in Chile. The last national survey of food intake was performed in 1974 *(12)*. That survey divided the country in regions and included a socioeconomic analysis of the population. Important differences were found in the consumption of protein according to income. More recent data can be found in the surveys of the National Institute of Statistics (INE), which conducts family budget surveys based on amount of money actually spent on different items. Surveys were performed in 1969, 1978, 1988, and 1997. Across socioeconomic levels, calorie consumption decreased in the first three surveys. However, it showed a remarkable increase in the 1997 survey. Table 1 shows an analysis of food consumption based on the 1988 and 1997 INE surveys *(18,19)*. Macronutrient intake increased in all income levels from 1989 to 1997, the increase being most pronounced for lipids (50% on the average). Although the average increase in calories was 25%, it was more pronounced in the poorer groups (quintile I to III). The same was observed in proteins and lipids, which is consistent with the higher prevalence of obesity observed in that group (*see* Section 4.).

The direct, positive relationship between income and food intake was also observed in smaller studies published in Chile. However, the pattern of food consumption in Chile is more confusing. The proportion of calories from fat has steadily increased and is in direct correlation with socioeconomic level, reaching almost 29% of calories in the richest groups. Even the poorest groups, which usually had intakes of fat below 25%, are already surpassing that limit *(20)*. Qualitatively, the foods consumed in Chile by the rich and the poor are essentially the same. Over time and throughout socioeconomic levels, the main contributors to calories have been bread and, to a minor extent, other cereals. It is estimated that average bread consumption in Chile in 1998 was 93.7 kg per person per year; however, in the rural zones, the mean intake was 140.8 kg per person

Table 1
Apparent Daily Consumption of Calories and Proteins Per Person, Chile 1988 and 1998

Quintile of income	*Calories*		*Proteins (g)*		*Lipids (g)*	
	1988	*1997*	*1988*	*1997*	*1998*	*1997*
I	1640	1961	44.5	56.3	42.2	56.7
II	1617	2083	43.3	60.2	43.3	62.8
III	1734	2143	47.4	61.0	47.5	64.8
IV	1988	2324	55.5	67.8	57.3	73.0
V	2200	2513	65.0	73.9	72.1	82.3
All	1869	2335	51.9	74.2	52.0	78.2

Based on food expenses in Greater Santiago ($n = 5076$ households for 1988 and 8445 household for 1998). (Adapted from ref. *18*).

per year *(21)*. In comparison, developed countries consume an average of 50 kg per person per year. Besides being a traditional part of the Chilean diet, bread is an inexpensive item that is widely used by the poorer sectors to satiate hunger.

The consumption of specific items in low-income populations can be determined as much by availability as by price. The choice of food available has usually been more limited in low-income than in high-income neighborhoods, where the presence of large supermarkets allows for a wider availability of different types of food.

Based on the recommendations to lower the risk of chronic diseases, the average Chilean diet could be considered a relatively healthy one. However, the differences in consumption trends between the different socioeconomic groups are substantial. The wealthier 60% of the population consumes a diet quantitatively different from the poorer 40% of the population. Additionally, the prevalence of obesity in poorer groups (*see* Section 4.) suggests a pattern of consumption or intrahousehold distribution of food that is not reflected in the average values given in the different studies. Nutritional studies that concentrate on these populations and that can accurately determine intrafamily variations are necessary to clarify the risks for these populations.

4. PREVALENCE OF OBESITY

Obesity is a nutritional disease with serious consequences. Its prevalence increases with age, and, although the increase with age has been accepted as inevitable, data show that this weight gain augments the risk of mortality *(22)*. The treatment of obesity is difficult and usually unsatisfactory, therefore its prevention is very important *(23)*.

It is well-accepted that obesity is a major risk factor for mortality, particularly from CVD *(24)*. An association has been found between obesity and hypertension, diabetes, lipid disorders, and ischemic heart disease. This condition is also related to other chronic diseases such as cholelithiasis, certain types of cancer, osteoarthritis, and pulmonary disease *(25,26)*. Therefore, obesity is a major public health concern that, if prevented, would certainly have a multiplying effect on the improvement of adult health.

Recent decades have witnessed a dramatic increase in the prevalence of obesity not only in developed countries but also in developing countries, including those of Latin America.

Table 2
Prevalence of Obesity[a] in Adults From Selected Latin American Countries[b]

	Men	*Women*	*Reference*
Brazil	8.2%	12.6%	*28*
Chile	15.7%	23.0%	*35*
Mexico	23.8%	42.3%	*30*
Peru	16.0%	23.0%	*32*
Paraguay	22.9%	35.7%	27

[a]Body mass index 30 kg/m^2 or greater.
[b]More details are given in the text.

Table 2 summarizes the prevalence of obesity observed in several selected countries of the Latin American region. Note that only in some cases is this prevalence at the national level, whereas other data are obtained from smaller studies that nevertheless involve a substantial number of individuals. There are countries in the region where the prevalence of obesity in adults and children has grown substantially, such as in Brazil *(11,27,28)* and Chile *(29)*.

A recent study of 3305 individuals (ages 35–64 yr) from Mexico City showed that in a 7-yr period (with no intervention), the total prevalence of excessive weight (BMI ≥25 kg/m^2) increased from 77.8 to 80%, and the prevalence of obesity (BMI ≥30 kg/m^2) increased from 26.61 to 34.7% *(30)*. In the same country in the city of Morelos, prevalence of obesity in the 11- to 24-yr age group was 21.2% *(31)*.

Brazil, the largest country in the region, has shown a steady decrease in malnutrition and an increase in the prevalence of obesity. A national survey was conducted in 1989 in Brazil using a representative sample of 14,455 households and was compared with a previous survey of 55,000 households performed in 1974–1975 *(11)*. An increase in prevalence was observed in both excessive weight and obesity in the period of time between the two surveys. Women were 2.5 times as likely as men to be obese, but the highest relative increase was seen in men, where prevalence almost doubled over 15 yr. In men, the prevalence was much higher in urban areas than in rural areas and showed a direct relationship with income. In both sexes, the more developed regions were associated with a higher prevalence of excessive weight/obesity *(11)*.

Compared with the survey in Brazil from 1989, data from the survey performed in 1997 show an increase in obesity in lower socioeconomic groups but a decrease in the prevalence of obesity in affluent women. Actually, this seems to be the only favorable change presently observed in the Latin American region *(28)*. In their analysis of different areas, the authors showed that in women, obesity was positively associated with income and negatively associated with education. Men from the same region showed only a positive association with income. In the more developed regions, prevalence of obesity was associated negatively only with education, whereas in men, it was positively associated with income. Recently in urban Peru, Jacoby et al. *(32)* also showed that in women, education was negatively associated with excessive weight and obesity *(32)*.

The prevalence of obesity is particularly high in Chile. Figure 6 shows the results of three studies of adults conducted in Chile. The studies from Berrios et al. took place in a population in Santiago in 1987 and 1992 *(33,34)*, whereas the third study was carried out in Valparaiso in 1998 *(35)*. All three studies were harmonized to cut-off points of a

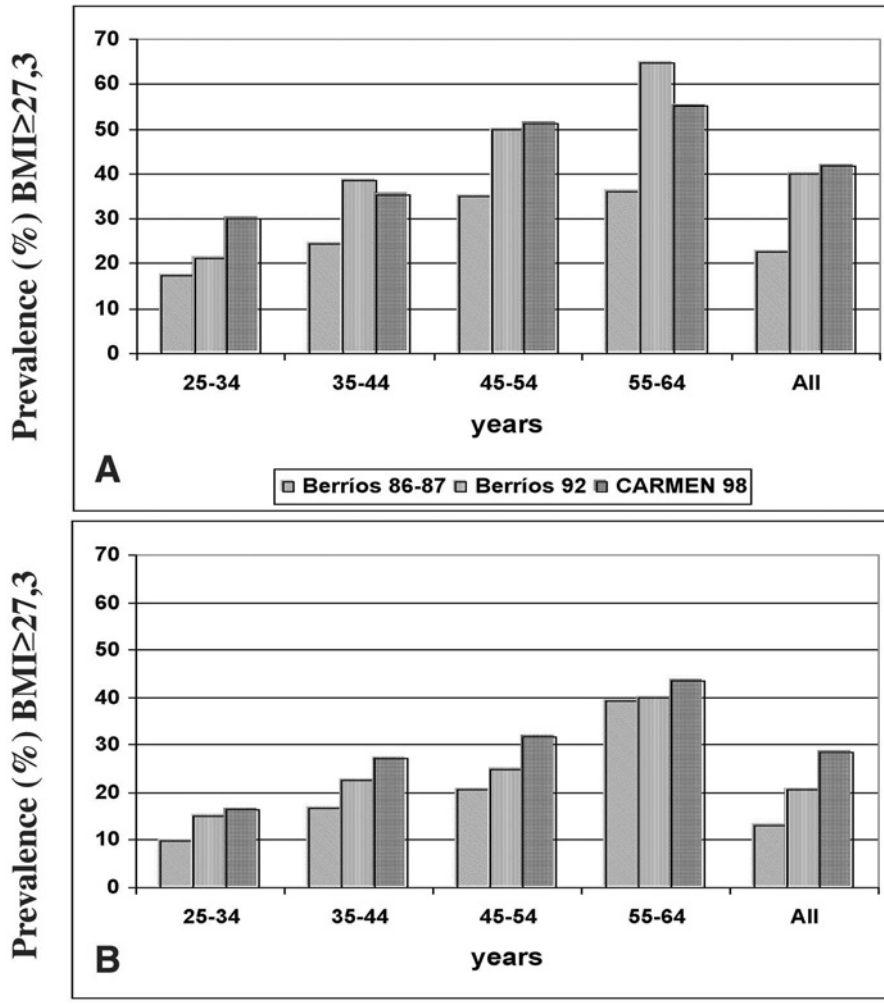

Fig. 6. Prevalence of obesity in Chilean women (A) and men (B). (Adapted from refs. *33–35*).

BMI >27.3 kg/m^2 for females and >27.8 kg/m^2 for males (the cut-off points used by Berrios et al.). The prevalence of excessive weight and obesity has increased with time and is almost consistently higher in women than in men; it shows an inverse correlation with socioeconomic level in women but not in men (data not shown).

The prevalence of obesity observed in Valparaiso in 1998 (measured as BMI ≥30 kg/m^2) was 15.7% in men and 23% in women. Furthermore, this study showed that 72.8% of males and 94.1% of females did not exercise in their spare time *(35)*.

Martorell et al. *(36)* reviewed the prevalence of obesity in nonpregnant women from Latin America and found that it ranged from 2.6% in Haiti to 12.1% in the Dominican Republic (Table 3). In all countries, urban women had a higher prevalence of obesity than rural women. Interestingly, a more recent study in Peru showed that 23% of women were obese *(32)*, indicating a remarkable increase in this condition from the 9.4% cited by Martorell in 1998 for this country *(36)*.

Table 3
Obesity Prevalence in Latin American Women Ages 15–49 Years[a]

Bolivia	7.6%
Brazil	9.7%
Colombia	9.2%
Dom. Rep.	12.1%
Guatemala	8.0%
Haiti	2.6%
Honduras	7.8%
Peru	9.4%

[a] Data from 1994–1996.
(From ref. *36*.)

A serious problem encountered currently is the remarkable prevalence of obesity in young children. The reasons for this are diverse. Nowadays, children from developed countries and from late-developing countries perform much less physical activity and tend to eat or drink high-fat, high-carbohydrate foods. For instance, in public schools in Chile, only 3 h of exercise are programmed each week. A study in Brazil showed that from 1974 to 1997, the prevalence of obesity increased from 4.9 to 17.4% and 3.7 to 6%, in children ages 6 to 9 yr and 10 to 18 yr, respectively; prevalence of obesity also increased from 4.9 to 18.4% in urban children *(37)*. Similarly, the prevalence of obesity in preschool children in Brazil has increased from 3.7 to 5.2% in the wealthier region *(38)*.

In Chile, the increase of obesity in children entering school has been notoriously rapid, rising from 6.5 to 17% in boys and from 7.8 to 18.6% in girls, respectively, between 1987 and 2000 *(20)*. De Onis et al. *(39)* published a review of obesity in preschool children in different countries of the world, including Latin America (Table 4). In the Latin American region, the highest prevalence of obesity was observed in Argentina, Chile, Peru, and Bolivia, whereas the lowest prevalence was observed in Haiti. Although it would seem that there is a correlation between obesity and GNP per capita, evidence does not support this conclusion, as seen in the case of Argentina, which has one of the highest GNPs in the region and a prevalence of obesity of 7.3% (the highest), and Bolivia, which has a prevalence of 6.3% and one of the lowest GNPs per capita in the area *(39)*.

What is the cause of such a high prevalence of obesity in preschool children? Although it is hard to establish the precise reasons for its increase, the prevalence of obesity seems to result from an increased amount of time spent in activities that do not require any effort (such as television watching or computer use) in detriment of regular physical activity. A recent study in Mexico showed that adolescents spend an average of 3.7 h/d watching television *(31)*, and at least 90% of Chilean school children have been reported to watch television for an average of 2 h on weekdays *(40)*. An extensive analysis of the literature showed that food is the most advertised product category in children's television, with most of the advertisements directed to sweetened products and fast food restaurants *(41)*.

Safety concerns of the parents regarding their children playing on the street certainly contributes the decrease in physical activity, although the attraction for stationary activities appear to be more important. Fortunately, programs are being developed that promote exercise, and preliminary results hint that they are successful (*see* the next section).

Table 4
Prevalence of Obesity (%) in Preschool Children From Selected Latin American Countries (W/H >95%)

Country	*Prevalence*
Argentina	7.3%
Bolivia	6.5%
Brazil	4.9%
Chile	7.0%
Colombia	2.6%
Costa Rica	6.2%
Dominican Rep.	2.8%
El Salvador	2.2%
Guatemala	4.0%
Haiti	2.8%
Mexico	3.7%
Nicaragua	2.8%
Panama	3.7%
Paraguay	3.9%
Peru	6.4%
Uruguay	6.2%
Venezuela	3.0%

(From ref. *39*.)

Another aspect that has often been blamed for the increase in the prevalence of obesity in children is the consumption of fast foods. One study in Europe even showed that there was a positive significant correlation between consumption of sugar-sweetened drinks and obesity in children *(42)*. This is worrisome because, for instance, marketing data indicate that Chile has one of the largest consumptions of soda per person worldwide *(43)*. In the United States, French et al. showed that in women and adolescents, fast food restaurant use is positively associated with energy, fat as percent of energy, and soft drinks and is inversely associated with the intake of fruits and vegetables *(44,45)*. In the same country, percent of total calories from food prepared away from home increased from 18 to 32% from 1977–1978 to 1994–1996, bringing an increase in the amount of total fat, saturated fats, and sodium *(46)*.

Nevertheless, it is hard to place the blame for the increase of obesity in Latin America mainly on the food industry. Rather, it is probably the whole environment (including lack of exercise, lack of nutrition education, and consumption of high-calorie foods) that is conducive to this condition. It is important to note that obesity in young children increases the probability of obesity in adult life. Obese boys and girls at age 1 yr have 21 and 20% probability, respectively, of being obese at age 35. However, if young men and women are obese at age 18 yr, the probability increases to 78 and 66%, respectively *(47)*.

The effect of early nutrition on the appearance of obesity at later years must be considered when discussing obesity. The studies of Barker *(48)* strongly suggested that early undernutrition resulted in increased adiposity and complications in later life. Stunting in children also has been shown to correlate with excessive weight *(49)*. Additionally, studies also revealed that children with heavy birth weights had greater chances of being overweight when they reached adult life *(50)*. All of these studies predict that, considering the

prevalence of undernutrition present in South America in recent decades, in the future we will be faced with a very heavy burden on health systems when these children reach adulthood.

The success of obesity prevention programs is very limited and they tend to be very labor-intensive and costly, which makes them very difficult to apply to the community. To prevent obesity, it is necessary to develop programs that combine intervention in nutrition education and promotion of exercise. A search of the Cochrane database for obesity prevention in children showed that there is scant high-quality data, and no general conclusion can be established. The chapter stresses the need for well-designed studies *(51)*.

5. CONCLUSION

The social and economic changes that are occurring in Latin American countries have a strong influence on the eating habits of the population. This has been correlated with an increase in the incidence of the chronic diseases. The prevalence of obesity in many countries of the region is reaching levels similar of those of industrialized countries. Nevertheless, in most cases, the available resources make it difficult to treat and prevent obesity. Obviously, the main aspects that have to be improved are nutrition education and the promotion of physical activity. The need to counteract the changes that are occurring in lifestyle and nutrition should be of utmost importance to governments, which should aim to create a level of nutrition literacy and a "consciousness" in the population toward appropriate food choices and physical activity. Only these will be able to maximize health benefits and minimize health hazards in the future.

6. PROGRAMS AND RECOMMENDATIONS

Given the variation in conditions in the different countries of Latin America, it is difficult to give general recommendations to prevent obesity. However, as economic development progresses in each country, all will eventually be faced with a wide range of problems in adults that will require attention. To diminish the expenses involved in this care, coherent prevention programs must be implemented to reduce the impact of diseases of "nutritional excess" on the health system and to improve the quality of life of the population as it ages *(52)*. An additional concern to remember is that the chronic, prolonged illness of an adult has an effect the health and well-being of the other members of the household *(53,54)*.

How do we tackle this problem? People that achieve economic progress increase their food-purchasing power, which correspond to an increased availability of food by industry. Although the role of food companies in health has long been debated, the reality is that their function (as viewed by their shareholders) is to make a profit. In reality, their advertisement budget is many times higher than what any government can spend on prevention. Only public education is capable of changing eating trends, and, to a substantial extent, food companies will respond to them. The influence of foreign food styles and imports in Latin American countries has been steadily increasing and will certainly continue to grow. As an example, a well-known international fast food chain increased the number of outlets in Chile from 1 in 1990 to 70 in 2002. This trend appears to be similar in other countries of the Latin American region. Consumption of prepared foods, either from large supermarkets or from fast food outlets, will be more prevalent because of convenience, price, or both. Popkin and Nielsen *(9)* analyzed the consumption of caloric sweeteners in 103 countries and observed a direct relationship between GNP and intake of sweeteners *(9)*. The authors

also showed trends in caloric sweetener intake in Americans ages 2 yr and older, which increased from 235 calories in 1977–1978 to 318 calories in 1994–1996. Calories from soft drinks increased 102% in the same period of time, from 52 to 105 calories/d.

We must consider special care when we propose programs to prevent obesity. In the United States, billions of dollars have been spent to prevent obesity, and, nevertheless, its prevalence continues to increase *(55)*. We are fighting not only environmental components such as availability of food at a low price but also natural components, because the signal to eat is stronger than the signal not to eat, brought about by millions of years of adaptation to a hunter–gatherer lifestyle *(23)*.

6.1. Current Programs

One of the few prevention programs currently present in the Latin American region promoting physical activity in Brazil is the Agita Sao Paulo experience *(56)*. This is an intervention program directed to promote physical activity in a community-wide intervention designed to increase knowledge about the benefits and the level of physical activity. The main message is that everyone should accumulate at least 30 min of physical activity of moderate intensity on most days of the week. Activities aren't encouraged in three settings: home, transport, and leisure time. The program is research-oriented and includes culture-linked social marketing with the inclusion of nonpaid media. There are more than 160 community groups involved, and the Brazilian government decided to make it a nationwide program (Agita Brazil). The impact of the program has reached outside Brazil, and similar programs have been implemented in other countries in Latin America.

An evaluation of the Agita program showed that the message reached 55.7% of the population; among these, 23.1% were aware of the main message *(56)*. Knowledge of the program's purpose was well-distributed among different socioeconomic levels, known by 67% of the most educated individuals. The prevalence of people reaching the recommendation was 54.8% (men: 48.7%; women: 61%), and the risk of being sedentary was smaller among those who knew the message (7.1%) compared with those who did not know (13.1%). Based on this experience, it appears that a multilevel, community-wide intervention to promote physical activity may produce good results as a prevention strategy.

An important aspect of this program is the political commitment. The Brazilian government has addressed the prevention of nutrition-related noncommunicable diseases through innovative legislative and regulatory actions, mass communications, and capacity building, all of which create a comprehensive approach for addressing poor dietary and activity patterns related to obesity in Brazil. The measures include labeling food appropriately, shifting the types of food purchased for the school food program, using mass media to communicate components of the food guidelines, establishing a smart shopping initiative, and training teachers and health workers. This represents an entire effort that took several years to institute *(57)*.

The Pan American Health Organization has implemented the project CARMEN, aimed at improving the health status of the population by reducing risks factors associated with noncommunicable diseases *(1)*. Regarding prevention of the behavioral risk factors in individuals, it focuses on smoking, unhealthy diet, physical inactivity, and obesity. The program develops partnership with countries in the region that diagnose the problem and sets up far-reaching interventions through the community, health professionals, and policymakers. Current partners of the network are Argentina, Brazil,

Chile, Costa Rica, Cuba, Colombia, and Peru *(1)*. To our knowledge, there is currently no thorough evaluation of the interventions.

Another experience in obesity prevention has been initialized in some Caribbean countries through the Lifestyle Project, developed by the Caribbean Food and Nutrition Institute *(58)*. This program is based on the assumption that healthy lifestyle habits should begin early in life to provide a lasting impact. The program is developed at the school level, and the general objective is to promote health and good nutritional habits in school children from the Caribbean. The objectives include the collection of information to guide the development and implementation of school-based intervention programs; the design of an intervention program for primary school children in urban and rural areas, with an emphasis on diet, physical activity, and positive self-esteem; the implementation of intervention programs to impart knowledge and skills necessary for practicing positive health behaviors; and the evaluation of the effectiveness of the program in modifying behavior.

The project Interhealth Chile (called Mírame) is an educative intervention program that promotes healthy lifestyles among school children *(59)*. It is based on the principles of the social learning theory, namely: (a) children learn by looking at and imitating other people; (b) children are influenced by the environment; (c) children need skills to develop and strengthen a healthy and positive concept about themselves; and (d) children need to develop social skills to face daily life. Although the program does not have a nutritional component, one is expected to be added in the near future. Some of the results from 9- and 10-yr-old children in the basal analysis showed a high rate of sedentary lifestyle, which increases with age. Other biological risk factors, such as hypertension and lipid disorder, were high for this age group and usually coexisted with the presence of obesity. The intervention has had very positive results regarding cessation of smoking and alcohol consumption, and it is considered a good educational strategy to promote other healthy lifestyles, such as adequate nutrition and physical activity.

Vida Chile is a program that concentrates on developing programs for healthy life and healthy environment *(2)*. It includes the participation of a wide range of governmental and nongovernmental Chilean institutions. The objectives of this program, directed to schools and the community, include the diminution of the prevalence of obesity and the promotion of exercise. When schools comply with the requirements, they become part of a network. Although the program was initiated a few years ago, it still has not had a thorough evaluation, which is expected soon.

The Obesity Program of the Catholic University of Chile is a university-based program. Although initially conceived as a treatment program, thousands of patients have received support from this multiprofessional approach that includes education and health promotion. A high prevalence of comorbidities associated with the patient population has been reported *(60)*. Although this type of program cannot be applied to the communities, prevention and treatment programs located at universities can be very useful regarding the training of adequate professionals to provide a better knowledge of the local reality and implement programs. However, the funds available for this type of activity are usually limited in developing countries.

6.2. Recommendations

Because of the limited resources available, an effective program of obesity prevention should be focused on "multiple-disease," and have the general goal of creating a

level of awareness that will guide individuals in the food choices they make in daily living. An effective program should involve all levels of education (starting in elementary schools—both public and private) as well as local health facilities and community groups. The program should also stimulate and welcome the participation of the food industry to improve labeling (for which regulations do not exist in the majority of countries in the region, to our knowledge), to diminish the use of food items known to have an undesirable effect on health, and to stimulate the production of food items that are healthy.

The program must be part of a coherent health and nutrition policy that has clear targets for promoting the concept of healthy nutrition *(61,62)*. The general framework of a prevention program can be defined by the following goals:

1. Develop a general awareness of the problem.
2. Identify those groups that are at risk in the public.
3. Remove or add those factors that affect the development of the disease (e.g., to instruct people on the consumption of saturated fats [remove], to promote physical activity [add]).
4. Treat those individuals in whom the disease is already present.

The goals of a particular program should be clearly defined and attainable, taking into account the different socioeconomic groups and the realities of daily living. This is an important aspect because the implementation of many programs that have been described as effective are much too expensive for the health system to apply at the national or local level. The goals and objectives outlined here concentrate on two major areas: nutrition education and the promotion of exercise. Regarding cost–benefit, these seem to be the most advantageous for developing countries.

Although a previous diagnosis is important, the high prevalence of obesity in most countries of the Latin American region calls for initiation of prevention programs even before a complete diagnosis is made. There is enough available information that clearly indicates the burden that this disease imposes on the health system; therefore, it is imperative that well-formulated programs be implemented immediately.

Several programs and recommendations were used to formulate the goals and specific objectives outlined here. These include the North Karelia Program in Finland *(62,63)*, the Multiple Risk Factor Intervention Trial in the United States *(64,65)*, and the Honolulu Heart Program in Hawaii *(66)* as well as the recommendations of the National Academy of Sciences *(67)*, the Agita Program in Brazil *(56,57)*, and others *(68–72)*.

6.2.1. Nutrition Education

The nutrition literacy of Latin Americans is low. An education program directed to the general population in each country can create an awareness directed to the proper food choices. The vehicles for the program should include:

1. Posters disseminated in public places like health clinics and hospital waiting rooms, government offices, schools, banks, pharmacies, public transportation, and other places where people have the opportunity to read announcements.
2. Nutrition education leaflets with simple and specific messages for distribution through health services, voluntary organizations, and schools.
3. Articles in newspapers.
4. Radio announcements of short duration (1–2 min) dedicated to a particular subject (e.g., high-calorie foods).

5. School programs to provide nutrition instruction to the students and to transfer the message to the family (e.g., in the North Karelia Project, school children gave their fathers a Father's Day card with a health message).
6. Television "shorts" with specific messages and development of specific television programs.

Although the costs of some of these components can be very high, they can be diminished by enlisting governmental and nongovernmental organizations (e.g., government-owned radio and television stations and private media as a public service).

The goals of this program should be as follows:

1. Increase awareness of the relationship between diet and heart disease, high blood pressure, diabetes, certain cancers, and other conditions.
2. Increase awareness regarding the ideal range of body weight and provide information on sound weight control strategies.
3. Provide information and education on healthy food choices at the point of purchase, home, the workplace, and school.
4. Provide information on selection and preparation of healthy diets.
5. Stimulate the food industry to make a wide choice of low-fat, low-sodium processed foods available to the public and to develop clear labels for these products. This also involves the establishment of government policy in terms of food labeling.

Although the time frame can vary according to the situation of each country, the specific objectives should be as follow:

1. The proportion of the population that can identify the principal dietary factors related to chronic disease should be at least 60%.
2. At least 75% of the schools should be able to provide sound nutritional education in their curriculum.
3. At least 60% of adults should be able to identify foods that are low in sodium, low in saturated fats, high in calories, and good sources of fiber.
4. At least 75% of adults should be able to understand the concept of losing weight by reducing calorie intake, increasing physical activity, or both.
5. At least 75% of adults should be able to read nutrition labels to make nutritious food selections.
6. At least 60% of adults should have at least a basic idea of the food guidelines and should make an effort to increase the intake of complex carbohydrates and fiber-containing foods.
7. At least 50% of the population should be able to maintain a fat consumption below 25% of calories, and saturated fats should be less than 10% of calories. (Although a substantial proportion of the population in the region seems to be at these levels already, there is a trend to increase fat consumption in the region, as indicated earlier.)
8. At least 50% of home food preparers should know not to add salt during preparation, and 75% of the individuals should know not to add salt to food before tasting it.

6.2.2. The Promotion of Exercise

The vehicles to be used in a program of exercise promotion are very similar to the ones indicated earlier. One strategy that produces good results is to enroll a sport personality (for instance, a soccer player) as the visible spokeperson for the program.

The goals of this program are as listed here:

1. Stimulate government to develop a policy directed toward developing a coherent exercise policy to stimulate exercise in children and adults.
2. Provide safe environments and appropriate facilities for exercise.
3. Incorporate in the school curriculum a sound physical activity program with the participation of teachers and parents.
4. Involve the community in activities and to transmit the concept that moderate exercise is beneficial in the prevention of coronary heart disease, hypertension, and obesity.

The specific objectives of this program should be as follow:

1. Incorporate physical activity as an integral part of the curriculum of at least 80% of all public and private schools.
2. Increase the proportion of people that engage in at least 30 min of moderate exercise per day to 60%.
3. Increase the proportion of overweight people that are engaged in some form of physical activity combined with appropriate dietary practices to achieve ideal body weight to 50%.
4. Increase the proportion of people older than age 20 yr who are aware that physical activity reduces the risk of heart disease, helps maintain appropriate body weight, retards the development of osteoporosis, and enhances self-esteem to at least 50%.

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32 Effects of Western Diet on Risk Factors of Chronic Diseases in Asia

Kaichi Kida, Koji Takemoto, Sei Won Yang, and Supawadee Likitmaskul

KEY POINTS

- The high-fat "Western diet" has caused an increase of childhood obesity in Asia.
- Hypercholesterolemia and type 2 diabetes in children are associated with obesity.
- Low-fat and well-balanced Asian diets are recommended for children.
- School programs are effective lifestyle interventions against risks for chronic diseases.

1. INTRODUCTION

Infectious diseases and malnutrition were the major health problems in most Asian countries before World War II and still are today in some parts of Asia. The structure of diseases changed dramatically in many Asian countries after World War II, in parallel with a rapid development of the economy followed by Westernization of lifestyles. From 1970 to 2000, the gross domestic product increased by 23 times in Japan, 52 times in Korea, and 17 times in Thailand, and lifestyles regarding housing, clothing, eating habits, and physical activities were Westernized in these countries. The Westernization of diets is demonstrated in an increase of protein and fat consumption at meals and the prevalence of American-style fast foods, such as hamburgers and fried chicken.

In Japan, Korea, and Thailand, the three leading causes of death today are neoplasms, heart disease, and cerebrovascular disease, similarly to Europe and North America. The cost of cardiovascular diseases (CVDs) and diabetes, which are closely related with lifestyles, was US$60 billion in Japan in 2001, accounting for 7.3% of the national budget. These chronic diseases, which often the result of lifestyles—particularly the diet—are not only a medical but also a growing economic problem in Asian as well as Western countries. Therefore, more attention is required to prevent risk factors for these lifestyle-related chronic diseases through nutritional intervention.

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This chapter reviews the relationship between Westernization of diets and lifestyle-related chronic diseases or their risk factors in Asian countries. Nutritional recommendations are described regarding prevention of risk factors for chronic diseases that often result from lifestyle choices.

2. EPIDEMIOLOGY OF CHRONIC DISEASES AND THEIR RISK FACTORS

2.1. Chronic Diseases

Heart disease, which develops from atherosclerosis and/or hypertension, has been increasing linearly each year and recently has become one of the major causes of death in Japan, Korea, and Thailand *(1)*. Although the death rate from ischemic heart disease (IHD) has increased several-fold in Japan and Korea in the last 20 to 50 yr, it is still substantially lower than in Europe and North America (9.9/100,000 in 1950 to 56.4/100,000 in 2001 in Japan *[2]* and 2.2/100,000 in 1983 to 12.5/100,000 in 1992 in Korea; *see* Fig. 1 and ref. *3*). In Thailand, the death rate from heart disease increased from 40.3 per 100,000 people in 1987 to 56 per 100,000 in 1992, a period of only 5 yr. The prevalence of IHD in one serially studied Thai community increased from 7 per 1000 in 1976 to 17 per 1000 in l983 *(4)*.

Cerebrovascular diseases also are emerging as a leading cause of death in Japan and other Asian countries (*1*; Fig. l). Although the death rate from total cerebrovascular diseases in Japan has gradually decreased during the past 30 yr, the death rate from cerebral infarction and its contribution to total cerebrovascular diseases have greatly increased over 50 yr (from 4/100,000 in 1950 to 65.3/100,000 in 2001; *see* Fig. 1 and ref. *2*). In Korea, the death rate from cerebrovascular diseases increased from 65.4 per 100,000 in 1983 to 80.4 per 100,000 in 1992 *(3)*. In Thailand, the death rate from hypertension and cerebrovasular diseases increased from 12.8 per 100,000 in l987 to 16.9 per 100,000 in 1992, and the prevalence of hemiplegia as a result of cerebrovascular diseases in one town increased from 1.5 per 1000 in l976 to 6.6 per 1000 in 1983 *(4)*.

Type 2 diabetes is caused mostly by insulin resistance (IR) associated with obesity and it is steeply increasing in many parts of the world, particularly in Asian countries, in parallel with an increase of obesity resulting from Westernization of lifestyles regarding eating habits and physical activities. The total number of patients with type 2 diabetes worldwide was estimated to be 133 million in 1995 and is anticipated to be 300 million in 2025, although its will occur more rapidly in Asia than in Europe and North America *(5)*. In Japan, the number of patients with type 2 diabetes whose HbA1c was 6.1% or higher or who were on treatment of type 2 diabetes increased from 6.9 million in 1997 to 7.4 million in 2001 *(6)*. Type 2 diabetes (a major type of adulthood diabetes) was believed not to develop in childhood; however, it is emerging rapidly in children in some ethnic groups, including Asian populations, Native Canadians, Native Americans, African-Americans, and Hispanics, who are known to be genetically more insulin-resistant than Caucasians *(7–14)*.

In Japan, all school children ages 6 to 15 yr are screened for diabetes every year with a urine test according to the Law of School Health; data obtained from the diabetes screening program for school children have revealed a steep linear increase of type 2 diabetes in school children in parallel with an increase of obesity of this age group in Japan (*see* Fig. 2 and refs. *7* and *15*). Childhood type 2 diabetes has increased by 2.5 times over the past 25 yr, and its incidence is now significantly higher than that

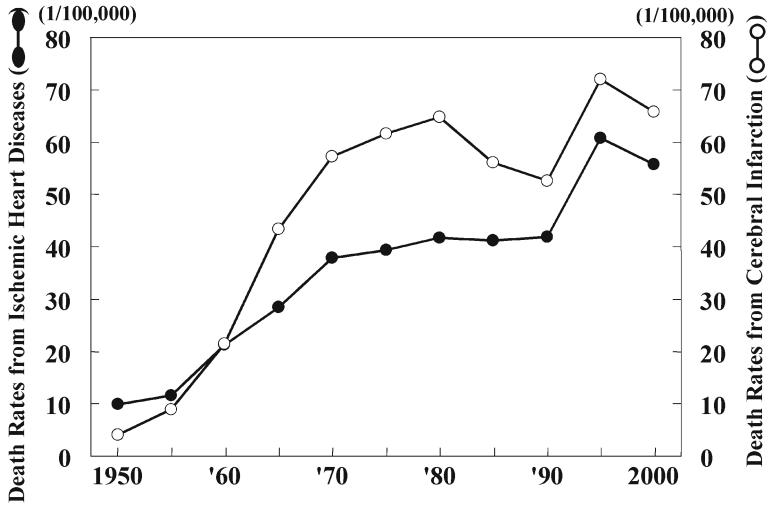

Fig. 1. Yearly change in death rates from ischemic heart diseases and cerebral infarction in Japan.

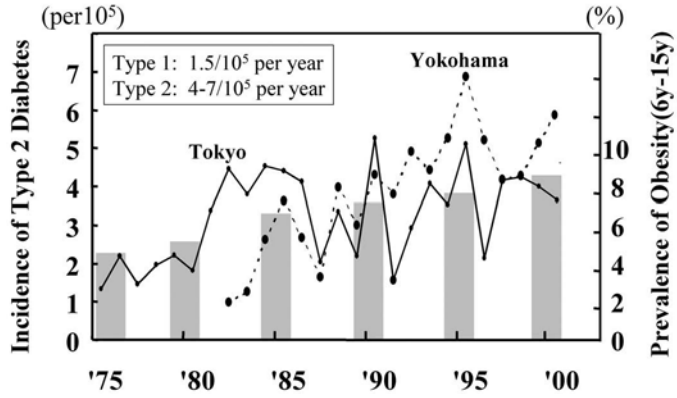

Fig. 2. Incidence of type 2 diabetes in Japanese school children.

of childhood type 1 diabetes in Japan (5–7/10^5 vs 1.5/10^5 per year) *(7,16–18)*. Similarly, in Thailand, childhood type 2 diabetes (newly diagnosed in Siriraji Hospital, the biggest hospital in Bangkok) increased from 5.9 cases per year between 1987 and 1996 to 15.9 cases per year between 1997 and 1999 *(19)*. In Singapore, childhood type 2 diabetes (newly diagnosed in the second biggest hospital) was five times greater over the past 3 yr *(20)*.

In New Zealand, type 2 diabetes was reported to account for 35% of diabetes diagnosed before age 30 yr among Northland Maori, whereas in previous studies in the

1980s, the majority of young New Zealand Maori patients had type 1 diabetes; this change indicates an increasing rate of type 2 diabetes in young New Zealand Maori *(21)*. Similarly, in a 5-yr follow-up study with Australian Aborigines, it was reported that the prevalence of impaired glucose tolerance and type 2 diabetes was 1.4 and 1.4%, respectively, at a mean age of 13.3 yr and 8.1 and 2.7%, respectively, at a mean age of 18.5 yr after 5 yr of follow-up *(22)*. Therefore, it is evident that rapid Westernization of lifestyles, particularly eating habits that increase fat intake, has magnified IR and caused an epidemic of type 2 diabetes in children as well as in adults among these ethnic groups. Type 2 diabetes should be recognized as one of the important health problems of children in these ethnic groups, because chronic complications such as retinopathy and nephropathy develop in childhood type 2 diabetes as fast as or even faster than in childhood type 1 diabetes *(23–25)*.

2.2. Risk Factors

Both hypercholesterolemia and hypertension are major risk factors for IHD and cerebrovascular diseases among Japanese people, similarly to Caucasian populations *(26,27)*. Nationwide surveys demonstrated that serum total cholesterol levels increased by 10–35 mg/dL between 1960 and 2001 in both adults and children in Japan (Table 1; Fig. 3; refs. *28–32*). Currently, the average total cholesterol levels of Japanese adults are still lower than those of American adults, but those of Japanese children are now similar or even slightly higher than those of American children *(33)*. All school children ages 9 to 12 yr (approx 10,000 school children) have been screened for serum cholesterol, blood pressure, and obesity in the town of Matsuyama every year since 1989. Their serum total cholesterol levels have increased by 10 mg/dL over 15 yr. The prevalence of hypercholesterolemia (>220 mg/dL) is 6.7% (343 of 5085) in school children ages 9 to 10 yr and 3.9% (174 of 4414) in those ages 12 to 13 yr in Matsuyama; their average total cholesterol levels are 176 ± 28 mg/dL in boys (n = 2619) and 177 ± 27 mg/dL in girls (n = 2466) at age 9–10 yr and 166 ± 27 mg/dL in boys (n = 2309) and 171 ± 27 mg/dL in girls (n = 2105) at age 12 to l3 yr *(7)*.

The prevalence of hypertensive diseases (hypertension and diseases from hypertension) in Japan increased from 8.0 per 1000 people in 1965 to l5.6 per 1000 in 1975 and 30.7 per 1000 in 1985 *(34)*. In 1990, the prevalence of hypertension among adults age 30 yr or older was 29.7 and 26.2% in men in women, respectively *(31)*. Among school children, the prevalence of hypertension was l9 per 1000 in boys and 13 per 1000 in girls ages 9–10 yr and 18 per 1000 in boys and 13 per 1000 in girls ages 12–13 yr *(7)*. It is well-established that obesity is associated with hyperlipidemia and hypertension among Japanese people *(35,36)*. The average body mass index (BMI; weight:height^2) among Japanese adults has increased during the past 40 yr, and estimated prevalence of adulthood obesity (BMI > 30) in Japan was 2.1 and 3.1% in men and women, respectively, in 1994 and 2.8 and 3.2% in men in women, respectively, in 2002, which is still lower than that in Western countries *(32,37)*. Nevertheless, the prevalence of obesity (20% overweight or more) among Japanese school children has increased remarkably (2.5 times) during the past 30 yr (*see* Fig. 4 and ref. *38*).

School children with hypercholesterolemia or obesity in Matsuyama are provided with health education, including diet and exercise recommendations, by school dietitians and school nurses. Significant improvement of serum total cholesterol or obesity is achieved in more than 50% of children after this instruction (*7*; *see* Figs. 5 and 6, Table 2).

Table 1
Yearly Change in Serum Total Cholesterol Levels in Japan

	1960 (28)	*1970 (29)*	*1980 (30)*	*1990 (31)*	*2001 (32)*
Males					
30–39 yr	167 (n = 676)	188 ± 33 (n = 239)	192 ± 38 (n = 897)	196 ± 35 (n = 620)	202 ± 31 (n = 243)
40–49 yr	175 (n = 1043)	194 ± 45 (n = 1043)	197 ± 38 (n = 1533)	204 ± 37 (n = 788)	209 ± 38 (n = 366)
50–59 yr	175 (n = 878)	181 ± 45 (n = 623)	199 ± 39 (n = 1081)	200 ± 37 (n = 758)	204 ± 33 (n = 406)
60–69 yr	179 (n = 428)	169 ± 43 (n = 334)	193 ±40 (n = 594)	197 ± 38 (n = 674)	202 ± 32 (n = 515)
Females					
30–39 yr	187 (n = 396)	184 ± 42 (n = 184)	178 ± 34 (n = 591)	186 ± 32 (n = 992)	186 ± 30 (n = 522)
40–49 yr	192 (n = 325)	179 ± 36 (n = 377)	191 ± 39 (n = 705)	200 ± 36 (n = 1124)	201 ± 32 (n = 592)
50–59 yr	207 (n = 216)	194 ± 44 (n = 142)	211 ± 41 (n = 735)	218 ± 37 (n = 995)	221 ± 36 (n = 684)
60–69 yr	194 (n = 104)	189 ± 44 (n = 33)	213 ± 39 (n = 597)	223 ± 38 (n = 870)	216 ± 34 (n = 691)

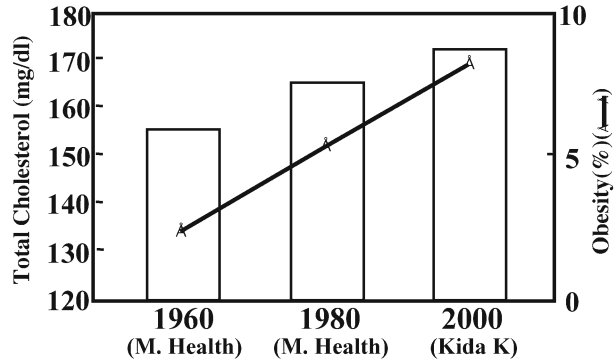

Fig. 3. Change in serum total cholesterol levels in Japanese children.

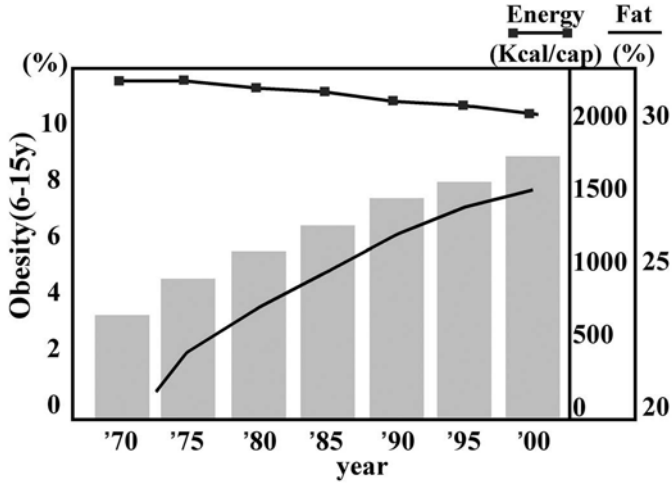

Fig. 4. Nutrition and childhood obesity in Japan.

This suggests that school-based programs for motivation and education play an important role in reducing risk factors for lifestyle-related chronic diseases that occur later in life.

In Korea, the average total cholesterol levels of children are comparable with those of Japanese or American children, and the prevalence of hypercholesterolemia (>200 mg/dL) among school children is reported to be 5–23% *(39)*. The prevalence of obesity has increased by 70% in boys and by 35% in girls over only 4 yr, from 1984 to 1988; these rates are now 12.4% among boys and 11.6% among girls in Seoul and Cheju *(40,41)*. In Thailand in 1985, a survey of coronary risk factors and nutritional conditions was performed among 3495 workers and reported that the prevalence of hypercholesterolemia (>200mg/dL), hypertension (>141/91) and obesity (BMI > 25) was 7l.3, 9.6, and 25.5%, respectively, in men and 65.4, 4.3, and 21.4%, respectively,

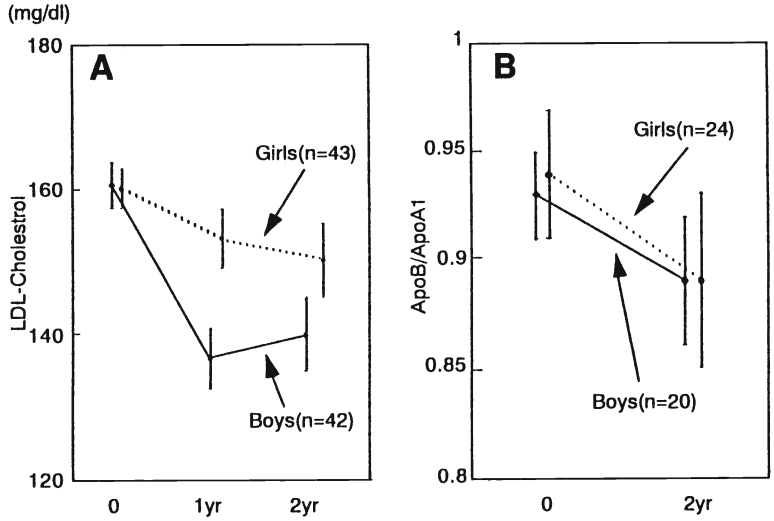

Fig. 5. Effect of intervention on LDL-cholesterol levels and ratio of apoB/apoA1. Children with 140 mg/dL or more of serum LDL cholesterol and those with an apoB/apoA1 ratio of 018 or higher were given nutritional education bu school dietitians and followed-up for 2 yr.

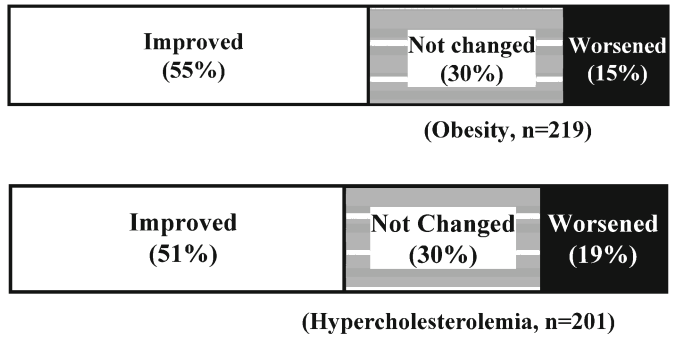

Fig. 6. Effect of school program on obesity and hypercholesterolemia in school children (2 yr follow-up).

in women. In 1991, another survey was taken among 519 hospital staff workers, who were expected to be more health conscious. This study demonstrated that the prevalence of hypercholesterolemia, hypertension, and obesity was 33.4, 4.6, and 18.2%, respectively, in men and 40.2, 1.8, and 27.0%, respectively, in women—lower than those in the former survey but still high *(4,42)*. Therefore, risk factors for lifestyle-related chronic diseases, including hypercholesterolemia, hypertension, and obesity, have increased over only a few decades in Asian countries and are now compatible with or even exceed those in Western countries.

Table 2
Effect of Intervention of Obesity Among School Children in Matsuyama (Longitudinal Study)

		Percent overweight	
		9–10 yr	*12–13 yr*
Intervention group			
Boys (n = 339)	33.0 ± 2 0.6	→	30.1 ± 0.9[a]
Girls (n = 261)	31.0 ± 0.7	→	23.4 ± 1.0[b]
Nonintervention group			
Boys (n = 82)	31.1 ± 1.3	→	33.9 ± 1.6
Girls (n = 111)	31.2 ± 1.0	→	30.0 ± 1.6

[a] $p < 0.05$, mean ± SE.
[b] $p < 0.001$, mean ± SE.

2.3. Obesity and Insulin Resistance

It is well-established that IR causing type 2 diabetes, dyslipidemia, and hypertension is associated with obesity not only in adults but also in children *(7)*. The homeostasis model assessment of IR (HOMA-R) is a conventional index for assessment of IR that is calculated by a formula of fasting blood glucose (mg/dL) × fasting serum insulin (μunit/mL)/450. The IR assessed by HOMA-R is highly correlated with BMI ($r = 0.77$; $p < 0.01$) or percent body fat measured by the bioimpedance method ($r = 0.88$; $p < 0.01$) in children (unpublished data), which suggests that IR develops according to progression of obesity in children, similarly to IR development in adults. It was recently discovered that the IR associated with obesity is regulated by the adipocytokines secreted by the fat cells in the adipose tissues tumor necrosis factor-α and resistin, which induce the IR, and adiponectin and leptin, which suppress the IR. Obesity (as expressed in BMI or percent body fat) associates with the decreased level of serum adiponectin ($r = –0.31$; $p < 0.01$ or $r = –0.32$; $p < 0.01$) and leptin resistance, shown by the increased levels of serum leptin ($r = 0.81$; $p < 0.01$ or $r = 0.88$; $p < 0.01$) in children (n = 109)(unpublished data).

3. NUTRITIONAL STATES

In Japan, the total energy intake per capita per day has not changed or gradually decreased for the past 40 yr (2098, 2184, 2119, and 1948 kkcal in 1950, 1965, 1980, and 2000, respectively; *see* Fig. 7; ref *32*). On the other hand, the percent energy intake from fat has increased 3.3 times over the past 40 yr (7.7, 14.8, 23.6, and 26.5% in 1950, 1965, 1980, and 2000, respectively). Intake of fat from animal, plant, and ash origin has increased by 4.2, 1.9, and only 1.7 times, respectively, over 35 yr, and the ratio of fat from animal, plant, and fish is now 4:5:1 in Japan (*see* Fig. 7; ref. *32*). The percent energy intake from saturated fatty acids (S), monounsaturated fatty acids (M), and polyunsaturated fatty acids (PUFA) in Japan was 2, 3, and 4%, respectively, in 1955; however, these percentages rose to 8, 9, and 9%, respectively, in 1985, which indicates that fat intake, particularly fat of animal origin, greatly increased over 30 yr *(43)*. The ratio of n-6 PUFAs/n-3 PUFAs increased from 2.8 in 1955 to 3.8 in 1985 *(43)* and 4.1 in 1990 *(44)*. The increase in the ratio of n-6 PUFAs/n-3 PUFAs might to the result of

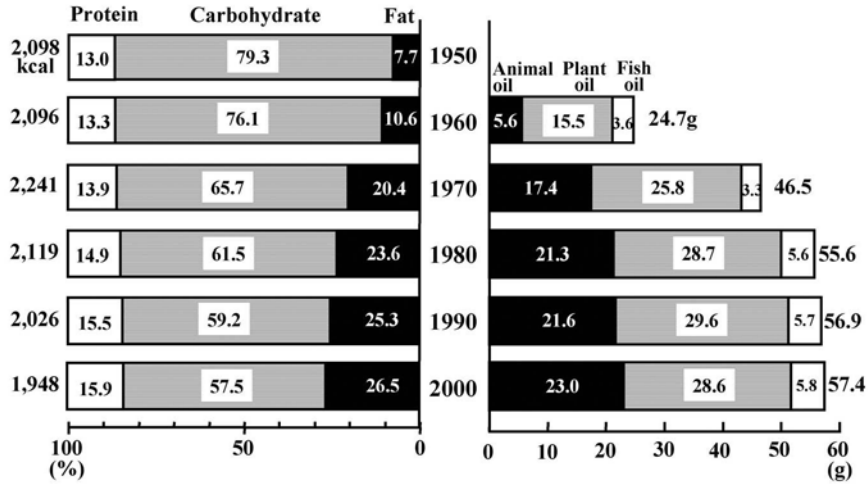

Fig. 7. Yearly change in energy distribution of nutrients and origin of intaken fat in Japan.

a relative decrease in intake of fat of fish origin and an increase in intake of plant oils containing a large amount of linoleic acid. In fact, the energy intake from linoleic acid increased 2.5 times, from 2.6% in 1955 to 6.4% in 1985 in Japan *(43,45)*. The protein intake per capita per day did not change in the past 30 yr in Japan, but protein of animal origin increased 1.7 times and protein of plant origin decreased by 34% during this period *(32)*. The change in the quantity and quality of nutrients might reflect Westernization of diets of Japanese people. Furthermore, occasions to eat meals outside at restaurants or even at American-style fast food bars, where fat-rich foods such as hamburgers and bed chicken are served, have remarkably increased in Japan during the past 30 yr (11.3, 16.9, and 18.5% in 1965, 1980, and 2001, respectively) *(32)*. Therefore, the eating habits of Japanese have been Westernized in quantity, quality, and manner.

A similar pattern is evident in Korea *(46)* (Table 3). Eating habits have been greatly Westernized during the past 20 yr. The total energy intake per capita per day has slightly decreased, whereas the percent energy from fat has increased threefold (5.7, 9.6, and 16.6% in 1971, 1981, and 1992, respectively) *(46)*. The ratio of fat from animal, plant, and ash was 19:69:12 in 1971 and 25:62:13 in 1990. The ratio of S:M:P was reported to be 2:2.8:1 and that of n-6/n-3 was 4.9 in 1990 *(46)*. The protein intake per capita per day was increased only 1.1-fold, whereas protein of animal origin increased 4.4 times from 1981–1992 *(46)*. The expense for fast foods and instant foods increased from 7.7% in 1973 to 12.9% in 1983, and occasions to eat meals outside the home also increased, from 2.8% in 1977 to 8.9% in 1986 *(46)*. In Thailand, Westernization of diets can be seen in increased fat intake by people in Bangkok. Fat currently accounts for 30% of total energy intake there *(46)*.

4. ATHEROSCLEROSIS AND NUTRITION

It is well-established that the origin of dietary fat and its composition of fatty acids are related to the risk of CVDs resulting from atherosclerosis. The epidemiological

Table 3
Yearly Change in Intake of Nutrients Per Capita Per Day in Korea

Nutrients	*1971*	*1976*	*1981*	*1986*	*1991*	*1992*
Energy, kcal	2072	1926	2052	1930	1930	1875
Carbohydrate, g	422	380	394	343	325	313
% Energy	81.5	78.9	76.8	71.0	67.4	66.8
Fat, g	13.1	20.0	21.8	28.1	35.6	34.5
% Energy	5.7	9.3	9.6	13.1	16.6	16.6
Protein, g	67.0	60.4	67.2	74.2	73.0	74.2
% Animal origin	11.6	20.2	32.2	41.2	42.7	46.6

study of Eskimos in Greenland and many other studies demonstrated that fish oils or n-3 PUFAs could play a role in preventing CVDs (*see* Chapter 9 and refs. *47–51*). Epidemiological studies of Japanese subjects showed similar results. The death rates from IHD and cerebral infarction were 2.6 and 9.2 times lower, respectively, in the town of Higashi-Izu, where people eat more fish than in Tokyo *(52)*. More recently, a large-scale epidemiological investigation was performed to determine the effect of eating fish on the risk of chronic diseases among adults in Japan. From 1966 to 1982, the 55,523 deaths out of a cohort of 265,118 were analyzed regarding their intake of fish. The study revealed that the total death rate and the death rate from cerebrovascular disease, heart disease, hypertension, and cancer were significantly lower in people who ate fish every day compared to those who did so sometimes, rarely, or never (Table 4; ref. *53*).

The degree of development of atherosclerosis in living subjects (measured by pulse wave velocity of the aorta) was 7.0 ± 1.1 m/s in a fishermen's village, where 90% of inhabitants ate fish every day and death from IHD was low. The pulse wave velocity was significantly slower (less sclerotic) than the value of 7.7 ± 1.3 m/s in a farmer's village, where only 11% of inhabitants ate fish every day and death from IHD was high *(54)*. Furthermore, renarrowing of coronary arteries after percutaneous transluminal coronary angioplasty for IHD was significantly decreased, from 37% in controls ($n = 43$) to 20% in subjects who were given 1.6 g/d of eicosapentenoic acid (EPA) orally for 3 to 4 mo ($n = 30$) *(55)*, as confirmed by a large-scale, double-blind investigation *(56)*. A multicenter study in Japan demonstrated that EPA was effective in arteriosclerosis obliterans (7.4 ± 1.87 mm in controls [$n = 25$] vs 6.0 ± 1.59 mm in subjects treated with 1.8 g/d of EPA for 6 wk [$n = 18$]), as assessed by the diameter of ulcers on the skin *(57)*. These data indicate that oils of fish origin or n-3 PUFAs could be beneficial in preventing atherosclerosis and related chronic diseases.

Another characteristic of the Japanese diet is a high intake of salt (NaCl), which likely plays an essential role in hypertension as an important risk factor for atherosclerosis. The current NaCl intake per capita per day in Japan is 12.2 g/d (i.e., 6.2 g/1000 kcal/d) and has not changed in the past 30 yr *(32)*. About 50% of total NaCl is from soy sauce, soy bean paste, and salted vegetables *(32)*. People in the eastern part of Japan consume 1.3 times more salt than those in the Kinki district, including Osaka and Kyoto *(32)*. Public education in a community for 8 wk successfully reduced NaCl intake of the residents by 3.8 g/d and lowered the systolic and diastolic blood pressure by 4.7 mmHg and 3.9 mmHg, respectively *(58)*.

Table 4
Cohort Study on Eating of Fish and Sex-, Age-Adjusted Death Rate (Relative Risk) From Chronic Diseases in Japan, 1966–1982

	Eating of fish					
Cause of death	*Every day*	*Sometimes*	*Rarely*	*Never*	x^2	*p*
Total death	1.0	1.07	1.12	1.32	9.13	<0.0001
Cerebrovascular diseases	1.0	1.08	1.10	1.10	4.54	<0.0001
Heart diseases	1.0	1.09	1.13	1.24	3.92	<0.0001
Hypertension	1.0	1.55	1.89	1.79	4.14	<0.0001
Liver cirrhosis	1.0	1.21	1.30	1.74	3.77	<0.0001
Stomach cancer	1.0	1.04	1.04	1.44	2.14	<0.05
Liver cancer	1.0	1.03	1.16	2.62	2.11	<0.05
Uterus cervix cancer	1.0	1.28	1.71	2.37	4.14	<0.0001
Observed l person year	1,412,740	2,186,368	203,945	28,943		

5. NUTRITIONAL RECOMMENDATIONS

Taking the rapid increase in lifestyle-related chronic diseases and Westernization of diets into consideration, much attention should be given to reduction of these chronic diseases by establishing a nutritionally appropriate lifestyle in Japan. The most recent recommendations appear in the sixth edition of *Recommended Dietary Allowance for Japanese* issued by the Ministry of Health and Welfare of Japan in 1999 (Table 5; ref. *59*). The recommended energy intake (E:kcal/d) was calculated by the formula:

$$\text{E(Kcal/d)} = \text{Standard Basal Metabolic Rate for Age (kcal/kg/d)} \times \text{Body Weight (kg)} \times \text{Intensity of Life (1.3–1.9)}$$

The recommended energy intake from fat is 45% of total energy intake for infants under age 6 mo, 30 to 40% for infants between 6 and 12 mo, 25 to 30% for young people ages 1 to 17 yr, and 20 to 25% for adults ages 18 yr and older. The recommended ratio of S:M:P among fatty acids is 3:4:3, and the recommended ratio of n-6 PUFAs/n-3 of PUFAs is 4.0, according to the results of analyses of the present nutritional conditions of Japanese people. The recommendation of S:M:P is in contrast to the previous recommendation, which was 1:1.5:1 *(60)*. The recommended intake of NaCl is 10 g/d or less and should be reduced to 7–8 g/d, if possible. There are a few arguments regarding these recommendations. There are no apparent reasons to elevate the upper limit of fat intake to 30% of the total energy for young people from 25% for adults. In fact, hypercholesterolemia is found in 8.2 to 9.6% of school children (Kida K, Matsuyama Study, 2003). Furthermore, pathological studies in Japan as well as in the United States revealed that atherosclerosis occurred with high frequency even in young people and was related to blood cholesterol levels *(61–63)*. Therefore, our study group of Matsuyama recommends that fat intake should not exceed 25% of the total energy in school children and adolescents ages 6–17 yr. Even infants, with the exception of babies and young infants, should not be exposed to fat-rich diets, because the period of infancy is critical toward setting eating habits in later life (Table 5). Although no definite consensus has been established regarding the ideal composition of fat and fatty acids, it is obvious from animal experiments and epidemiological studies that fat of animal origin

Table 5
Nutritional Recommendations in Japan, Korea, and Thailand

	Japan (59)	*Japan (Matsuyama)*	*Korea (64)*	*Thailand (65)*
Fat Intake (% total energy)	25–30 (1–17 yr) 20–25 (18 yr)	25–30 (1–5 yr) 20–25 (>6 yr)	20	>30
Fat intake from animal:plant:fish	4:5:1	3:4:2		
Fish meat (g)				
animal meat (g)		1.0–1.5		
S:M:P	3:4:3	1:1.5:1.5	1:1–1.5:1	1:1:1
n-6 polyunsaturated fatty acid/ n-3 polyunsaturated fatty acid	4	3		

should be reduced and the ratio of n-6 PUFAs/n-3 of PUFAs should be lowered as much as possible. It might be feasible for Japanese to take more fat of fish origin and less of animal origin while also reducing linoleic acid (n-6 PUFA) intake by avoiding oil or food fortified with linoleic acid; thus, the ratio of n-6 PUFAs/n-3 of PUFAs may be lowered to the level of 2.8, which was the ratio in 1955. Accordingly, our study group recommends that the ratios of S:M:P among fatty acids and of n-6 PUFAs/n-3 PUFAs be 1:1.5: 1.5 and 3.0–2.0, respectively (Table 5). Regarding salt intake, the recommended intake of salt (10.0 g/d) is still high compared with those in many other parts of the world. We recommend salt intake to be 7–8 g/d (3.5–4.0g/1000 kcal) for adults and children above age 6 yr.

In Korea, the recommended fat intake is 20% of total energy intake and recommended ratios of S:M:P and n-6 PUFAs/n-3 PUFAs are 1.0:l.0–1.5:l.0 and 4–10, respectively *(64)*. In Thailand, it is recommended that the fat intake should not exceed 30% of total energy intake, with equal distribution of S, M, and PUFAs among fatty acids *(65)*.

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IX Critical Issues for the 21st Century

33 Alcohol
The Balancing Act

William E. M. Lands

KEY POINTS

- Excessive alcohol consumption contributes to 4 of the 10 leading causes of death in the United States.
- Alcohol consumption is split, with 33% of the population consuming 95% of the alcoholic beverages and 33% abstaining. The US population median intake is much less than the average (mean) intake.
- An increase in average alcohol intake could increase the harm to the vulnerable subgroup that seems to be a predictable portion of the population.
- One alcohol drink per day gives little detectable blood alcohol for 22 of the 24 h in a day, leading to uncertain mechanisms for hypotheses regarding its long-term benefit.
- In 2000, exposure to alcohol caused 3.2% of deaths and 4% of disability worldwide.
- The dramatic difference in deaths resulting from ischemic heart disease in France (compared to the United States and England) is much less when combined with deaths from alcohol-associated causes.
- A causal role for alcohol in decreasing cardiovascular mortality has not been proven.

1. INTRODUCTION

Intakes of nutrients by individuals are customarily described with a broad range of acceptable choice between some average required lower limit and an average safe upper limit. Safe upper limits of average alcohol consumption by individuals have been a principal focus of national dialogue for 200 yr, and moderation has been repeatedly urged. The Dietary Guidelines for Americans *(1)* cautions against more than one drink per day for women or two drinks per day for men (with a standard drink being either 12 oz of regular beer, 5 oz of wine, or 1.5 oz of distilled spirits, each of which contains about 14 g [100 calories] of ethanol). The Dietary Guidelines changed wording from 1980 to 2000 to give more clear advice and cautions *(2)*. Presently, no major scientific body recognizes a nutritional need for alcohol nor recommends that those who do not drink should begin to do so.

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The widely discussed hypothesis that moderate alcohol intake might decrease cardiovascular death rates prompted this review of evidence to guide future preventive nutrition advice. A useful context is in balancing possible benefits and risks. This chapter examines the risk for developing alcohol use disorders by individuals who we are still unable to identify *a priori* against *the* proposed benefits from drinking alcohol. The risk of alcohol disorders has a long history of inadequate intervention, and the possible benefit of less cardiovascular mortality has uncertain proof. Insufficient evidence about the many mechanisms for cardiovascular disease (CVD) processes parallel insufficient evidence on how alcohol may diminish those processes—or even whether alcohol actually diminishes CVD. The onset and progression of CVD is not caused by a deficiency of alcohol intake that needs to be corrected. Rather, the chronic nutritional imbalances that exacerbate inflammatory atherogenesis, myocardial arrhythmia, and platelet thrombosis are rational targets for primary and secondary prevention. Readers interested in designing preventive nutrition interventions or in making personal decisions based on a fully informed choice will find that the slow progress in defining precise terms and in acquiring evidence of causal mechanisms *(3)* makes setting policies seem premature at this time. The information assembled here does not form a smooth, solid series of proofs but, rather, presents key concepts arrayed as beads on a string for the reader's contemplation.

2. BALANCING CAUSES AND CONSEQUENCES

2.1. The Importance of Contexts

Causes and consequences are the makings of science. They are the way that scientists interpret events by converting instinctive questions of purpose (i.e., "Why?" into the dimensions of "How come?" and "So what?"). By balancing new controlled information about things and events with earlier accumulated associations and memories, we learn to understand life and our surroundings. Experience provides each of us with a new balance of positive and negative associations from which our mind produces conscious and subconscious outcomes that shape our interpretations, emotions, and actions. That balance is the context within which new evidence is assimilated to create the cognitive and emotional significance of that information for each individual. Each of us believes that we know some things with certainty and that we can use that knowledge to choose a rational course of action. This chapter examines some of that knowledge in a context that can aid informed choices.

Scientific procedures are designed to systematically assemble, within a defined context, the evidence needed to convert conjectures into conclusions. When possible, we design controlled interventions to gain certainty about how an event can directly cause a consequence. There is a constant challenge in such experiments to recognize which evidence proves a direct cause–effect link, which evidence reflects a secondary consequence, and which evidence marks an unrelated associated event. All three types of information occur in an uncertain balance as the observational science of epidemiology collects and records diverse events associated with alcohol consumption. The strength of epidemiological observation is in its ability to define boundaries of a problem and to prompt new hypotheses about causes. However, testing those new hypotheses requires interventions in the context of well-defined terms and well-designed positive and negative control measurements. These are often hard to obtain with volunteer human subjects.

In balancing information on preventive nutrition and the possible benefits and risks of drinking beverage alcohol, we need to consider information of an association that is suitable for suggesting a hypothetical cause separately from information of a controlled intervention that can prove a causal relationship. However, even careful considerations are confounded by heterogeneity among diverse individuals in society and by heterogeneity in perception of the severity of alcohol-related risks.

2.2. Individuals Within Populations

Human life is the complex consequence of about 35,000 genes producing products in different amounts at different times in response to gene–gene and gene–environment interactions. Although the genomic sequence for each individual is finite, adaptations produced from interactions between the 35,000 genes and the remaining 95% of the human genome include such a vast number of possible outcomes that they are completely unpredictable in detail. Imprecision is further assured by another layer of complexity regarding neuronal adaptation during learning and memory storage. Each thing we learn is through neural associations that involve thousands of synaptic links, and "learning from experience" inevitably differs for each individual. As a result, the combined associative memories that link events together have a different balance of cognitive and emotional valence for each individual. This leads to diverse interpretations when attempting to answer complex questions like "Is drinking alcohol beneficial or harmful?" and "Why do some people drink so much?" The diverse interpretations and priorities confound efforts at consensus regarding possible causes, and they require open evaluation in scientific dialog (discussed further in Section 6.). In fact, consensus may be further confounded because the questions truly have different answers for different individuals.

Above and beyond the complex adaptive response systems of the living body and the memories and emotions of the mind is an added level of complexity of collective societal adaptations to perceived problems. Democratic societies struggle to balance the preferences and priorities of diverse individuals within the overall heterogeneous society. Public health efforts in preventive nutrition seek to maximize the number of people who are free from harm and to minimize the degree of overall harm to the community. The efforts inevitably develop into conflict when balancing each individual's free choice with the effort to decrease perceived risk to the group. To develop a consensus for interventions, the physiological condition to be prevented and the causal mediators to be targeted need careful definition. However, statistical descriptions of an aggregate community do not precisely describe any individual within a heterogeneous community for which a balanced consensus is desired.

As epidemiologists gather more information on larger numbers of subjects to increase the certainty of an average association, they also increase the number of people who are not precisely described by the population mean value. Thus, a struggle for balance is evident in efforts to develop scientifically credible targets for acceptable community intervention that could also be credibly sound advice for individuals. The resultant compromise regarding possible causes of good and bad health is demonstrated in a nation's guidelines for healthy lifestyles. For example, the Surgeon General's Report on Nutrition and Health *(4)* described a situation and a state of knowledge in 1988 that is similar to that which exists currently: "Excessive alcohol intake is a prominent contributor to 4 of 10 leading causes of death in the United States." Also, the

Dietary Guidelines for Americans *(1)* are intended to meet nationwide nutrient requirements, promote health, support active lives, and reduce chronic disease risks by combining available, affordable, and enjoyable foods to create healthful diets that fit appropriate physical activity. The Dietary Guidelines for Americans recommends that "if you drink alcoholic beverages, do so in moderation," and defines "moderation" as noted in the Introduction.

A "healthy diet indicator" developed from World Health Organization dietary advice evaluated dietary patterns and mortality in different cultures *(5)*. For different regions (Finland, Italy, and the Netherlands), the 20-yr mortality was lowest in men who had the highest healthy diet indicator. The indicator was positively associated with alcohol intake and had a strong inverse association with mortality from CVD. It is important to decide which data define how much benefit should be allocated to each dietary and lifestyle factor. Do the data assign healthiness to the diet or the alcohol? An irony lies in the fact that the dietary energy provided by alcohol was set aside and not included in calculations for macronutrients as a percentage of energy intake *(5)*. In developing social policy, do alcohol calories count or not? This question echoes the longstanding uncertainty regarding how to interpret alcohol calories *(6)*, which is described further in Section 4.

2.3. Lifestyle Choices

A 6-yr longitudinal study of 43,757 male health professionals provided useful insight into clustered patterns of voluntary food choice *(7)*. When choices of these well-informed individuals were ranked by increasing quintiles of mean saturated fat intake (grams per day), there were corresponding progressively increasing gradients of mean saturated fat and total fat intake as percent of energy, servings of high-fat dairy food, and servings of red meat, whereas there were progressively decreasing gradients of servings of fruits, vegetables, and fish. Interestingly, the percent of individuals who smoked increased with higher quintiles of saturated fat intake, whereas mean physical activity and mean alcohol consumption decreased. The coupled lifestyle choices of these well-informed individuals create difficulty in unraveling the web of factors that contribute to the overall health status of each individual. If lifestyle choices cluster together in a healthy lifestyle, what evidence indicates the degree to which each variable was a causal factor for CVD mortality? Could attributing lower relative CVD risk to more alcohol intake be attributed to parallel choices favoring exercise, fruits, vegetables, and fish or to inverse choices of high-fat dairy, total fat, or smoking? In this regard, an earlier report on these subjects *(8)* noted that increased alcohol intake was also associated with an increased percentage of individuals who regularly used aspirin, an agent well-known to decrease thrombotic mechanisms and CVD mortality. This illustrates another lifestyle feature that might impact on the interpretation. The earlier report *(8)* showed a parallel rise in the percentage of individuals who smoked with higher alcohol intakes, whereas Ascherio et al. *(7)* reported opposing smoking and drinking gradients when subjects were stratified by saturated fat intake. Compared to consumers of other alcoholic beverages, wine drinkers tend to have a lower prevalence of smoking; higher education and household incomes; higher intakes of dietary fiber, potassium, vitamin E, and total carotenoids; higher amounts of fruits, vegetables, and grain products; and lower total fat intakes *(9)*. Conversely, beer and liquor drinkers had somewhat lower education and household incomes; higher rates of current smoking;

higher energy and total fat intakes; and consumed lower amounts of fruits, vegetables, and grain products. Such clustering of attributes illustrates the well-known need for caution in interpreting associative relationships in observational epidemiological data *(10,11)*. Controlled interventions are needed to provide a conclusive test of an independent causal role.

3. BALANCING DIVERSITY AND COLLECTIVITY

3.1. Medians, Means, and Moderation

Diversity among individuals is frequently reflected in measures of variance, such as standard deviations around a mean value that is commonly displayed in Gaussian bell-shaped distributions. Such distributions mold our concept of average attributes of average people. Familiarity with such curves leads us to easily use the bell-shaped paradigm as a first approximation in judging new situations with which we are unfamiliar. However, published patterns of alcohol consumption in many different communities approximate a skewed distribution that is similar to the pattern shown in Fig. 1. The figure schematically indicates that 33% of Americans drink 95% of the alcoholic beverages *(4)*, whereas about 33% abstain from comsumption of alcoholic beverages. Additionally, the average (mean) weekly intake of 10 drinks per week is much greater than the middle person's (median) intake of 1 to 2 drinks per week and much less than that for individuals in the upper 5% of alcohol intake, who may account for almost 50% of total alcohol consumption *(4)*.

Thus, a tendency to think of alcohol intakes in terms of a Gaussian distribution produces dissonance among people who are evaluating aggregate information on benefits and risks for a population of individuals. In this situation, concepts about "average" behavior or "average" consequences need careful and patient description to avoid misrepresenting the actual evidence. Although people may regard the mean value as that of an "average" person, the median value is probably closer to their intent. Social policy about "moderation" in an open democratic society might be expected to relate more to median rather than mean values. Serious ethical issues accompany the urging of median individuals to act like those at the mean.

3.2. Public Health Aspects of Collectivity

The skewed relationship of median and mean values creates another important public health question: "Will the incidence of heavy 'problem' drinking in a community be greater when the mean value is greater?" This question about collectivity gains importance as communities consider preventive nutrition advice that is based on putative benefits of moderate drinking on the risk of cardiovascular disease. Encouraging this tactic may cause median and mean alcohol consumption to rise if abstainers feel encouraged to begin drinking alcohol.

Reports of an almost lognormal distribution of alcohol consumption among adults in France *(12)* led to suggestions that some collective social mechanism may affect drinking patterns within a population, possibly linking an increase in heavy drinking with an increase in mean consumption. Subsequent studies confirmed a close relationship between mean and heavy consumption of alcohol for diverse populations *(13)*. Ledermann's lognormal hypothesis *(12)* provides a useful predictive rough estimate of the positively skewed distribution. A recent report examined health surveys for 32,333 adults

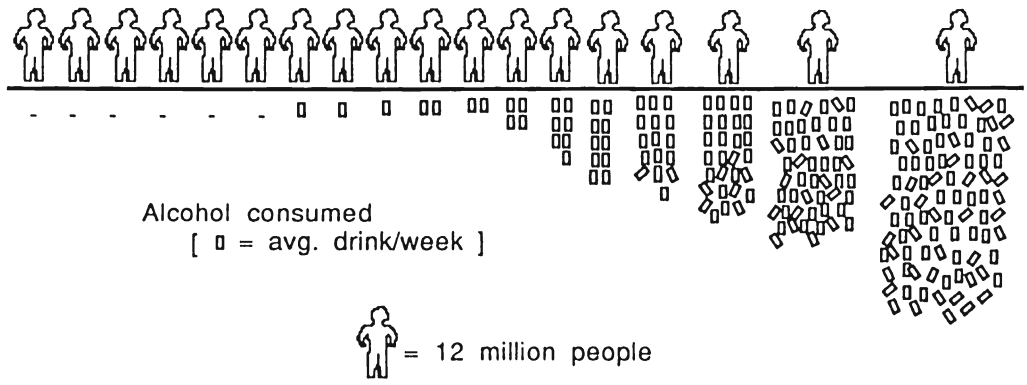

Fig. 1. Is alcohol everybody's problem? The figure represents results reported in The Surgeon General's Report on Nutrition and Health (1988). Each small rectangular symbol represents one standard alcoholic beverage (14 g of ethanol) consumed per week, and each human figure represents 12 million Americans age 14 yr or older. The population in July 1998 was about 270 million, of whom about 80% were 14 yr or older.

from 14 separate regions in England and found a strong positive association between mean regional consumption and the prevalence of heavy drinking *(14)*. The associations were similar for men and women, and they were similar in magnitude when the analysis was restricted to those under age 65 yr and when abstainers were excluded. Values for mean alcohol consumption by men in the different regions ranged from 15 to 22.7 units per week, with an English alcoholic beverage unit equivalent to 8 g of ethanol (contrasting with the 14-g standard in the US Dietary Guidelines). A 1-unit per week higher mean consumption of alcohol was associated with 6.3% more men drinking greater than 21 units per week. A serious implication for public health is that heavy drinkers may not be indifferent to the acceptability of drinking in their culture. The social determinants for abstention may differ from determinants for heavy drinking *(14)* in ways that do not permit assuming that adverse consequences of more heavy drinking will be offset by beneficial effects on CVD.

An alternate study of collectivity used data from the US National Longitudinal Alcohol Epidemiologic Survey (NLAES) with a sophisticated multiple linear logistical regression model fitted to major significant predictive variables to project possible outcomes of different public policies *(15)*. Different prevention strategies were compared to test their possible effect on the predicted prevalence of alcohol abuse and dependence in the population, and predictions were comparable for either the collective population or the high-risk individual strategy. A 25% reduction in average daily intake of all current drinkers reduced the predicted prevalence from 16.7 to 13%. Alternatively, a 28% reduction in average daily intakes of all drinkers who occasionally or usually exceeded the moderate drinking cut-point lowered the predicted prevalence to 12.6%. Finally, reducing the consumption of usually immoderate drinkers by 41% resulted in a 13% predicted prevalence of alcohol abuse and dependence within the drinking population. The authors concluded that reducing neither overall consumption nor high-risk consumption appeared to be superior to the other in reducing the prevalence of alcohol abuse and dependence.

Another important finding from the NLAES that had implications for prevention policies was that more than 40% of respondents who initiated drinking before age 15 yr were classified with alcohol dependence at some time in their lives *(16)*. Furthermore, the odds of lifetime alcohol dependence were 14% lower with each increasing year of age at first use. Efforts to prevent automobile accidents by limiting access to alcohol for adolescents who are newly introduced to driving skills and judgements can also be evaluated as a possible strategy to decrease the prevalence of alcohol use disorders. Is early onset a potentially modifiable risk factor in development of disorders or is it only a marker of an inevitable, possibly unmodifiable, development of alcohol use disorders? More controlled evidence needs to be evaluated to resolve these alternatives, and they should be resolved if preventive strategies are to be based on proven causal factors.

Another indication of a possible collective population influence exists in the strong positive association of mean apparent alcohol consumption in the 50 different US states *(17)* with two indices of heavy drinking: the prevalence of total ethanol-related disease and chronic liver disease in each state *(18)*. Similarly to the results from England, these results suggest that heavy drinkers may be a predictable portion of a population. Collectivity was also evident in the standardized data from the Intersalt Study. The Intersalt Study was an international, multicenter study of adults representing 52 populations in 32 countries that showed close and independent associations between the population mean and the prevalence of deviance for each of the variables examined: correlation coefficients were 0.85 for blood pressure, 0.94 for body mass index (BMI), 0.97 for alcohol intake, and 0.78 for sodium intake *(19)*. These findings imply that distributions of health-related characteristics increase and decrease as a whole. Therefore, an increase in average alcohol intake that is intended for the health and well-being of the general population could be responsible for increased harm to the vulnerable subgroup.

3.3. Temporal Variance in Alcohol Intake Patterns

Another feature of diversity in alcohol consumption that impacts on health outcomes is the pattern of intake within the period of time being used for comparisons. The biological impact of one drink per day for 7 d clearly differs from that of seven drinks on 1 d per week, although both lifestyles have mean intakes of one drink per day. Unfortunately, few epidemiological reports note such temporal variances in intake. Because aggregate data fail to give clear insight into the daily intensities of alcohol exposure that occur in individual cases, the credibility of general interpretations that are made from aggregate data may be compromised by that failure. To avoid that weakness, careful, systematic records of daily intake were obtained from 50 heavily drinking volunteers enrolled at the Vermont Alcohol Research Center who reported their alcohol intake for 112 d using a computer-automated interactive voice simulation system accessed by touchtone telephone through a dedicated 800 number *(20)*. Subsequent reports have confirmed the value of interactive voice responses in assigning valid patterns of individuals' alcohol intake *(21,22)*, an important aspect in avoiding inappropriate stereotypes of alcoholism *(23)*.

Results from 5151 self-reports of daily drinking *(20)* documented a wide variance, with somewhat greater drinking on weekends than weekdays (Friday = 5.7 ± 5; Saturday = 5.7 ± 4; Sunday = 4.2 ± 4; Monday = 3.6 ± 3; Tuesday = 3.5 ± 3; Wednesday = 4.2 ± 4; Thursday = 4.2 ± 3). Other data collected by traditional methods immediately after the study confirmed the expectation that subjects who drank more heavily

retrospectively under-reported their consumption significantly more than subjects who drank more lightly. A more detailed analysis of individual daily alcohol intake *(24)* was made for 12 subjects with comparable quantity and frequency of alcohol consumption: 6 lighter drinking alcohol-dependent and 6 heavy drinking nondependent subjects. Alcohol consumption by nondependent heavy drinkers had long-term variations (weeks or months, perhaps reflecting social occasions), whereas the dependent subjects had stable, characteristic 7-d drinking cycles. Alcohol drinking was rather homogeneous throughout the week in France, whereas Fridays and Saturdays accounted for 60% of total alcohol consumption in Northern Ireland *(25)*. Consumption of wine was higher in France, whereas consumption of beer and spirits was higher in Northern Ireland. Weekend drinking demonstrates the inability of mean aggregate values to describe the state of an individual at a given time. Average volume of alcohol consumption relates to many major disease outcomes in a detrimental fashion, and the pattern of drinking is an additional influential factor for coronary heart disease and injury. The influence of patterns of drinking may be underestimated because pattern measures have not been included in many epidemiological studies *(26)*.

Each person metabolizes alcohol in a fairly reproducible and consistent manner, with characteristic rising and falling curves of tissue alcohol levels *(27)* and most ingested alcohol apparently converted to carbon dioxide within hours. The transitory period of elevated tissue alcohol levels constitutes a controversial period within which scientists attempt to interpret how alcohol has its impact. With one drink per day, blood alcohol will probably be very low for 22 of the 24 h in the day *(28)*. Dietary guidelines emphasize drinking alcohol with meals *(1)*. Are benefits and risks of alcohol drinking linked to ambient alcohol levels in tissues, or are they linked to some associated aspect of metabolizing alcohol during prandial or postprandial states? These questions need to be addressed in explaining how alcohol consumption can cause the effects that are attributed to it.

Very low levels of alcohol may influence human physiology in ways not widely recognized, discussed, or even carefully documented. When preventing physiological signs of alcohol withdrawal, alcohol was infused intravenously (nearly equivalent to seven drinks per day) without elevating blood alcohol levels beyond that regarded as "nondetectable" *(29)*. More controlled analytical monitoring during similar intravenous treatment to prevent alcohol withdrawal *(30)* noted success when arterial levels were near 0.1 mg/mL (often regarded as "undetectable" with standard methods). However, treatment failed when the alcohol levels were essentially zero. To answer questions regarding how beneficial physiology results from alcohol levels near 0.1 mg/mL, we need to determine the still unknown causal mechanism by which such effects can be interpreted. Much evidence from controlled clinical interventions exists to support rationales or mechanisms for benefits caused by alcohol. Claims that one drink per day can prevent CVD need plausible mechanisms to gain credibility.

3.4. The Disease Concept of Alcoholism

During the 18th century, the balance in attitudes toward chronic drunkenness gradually shifted away from it being a voluntary choice that people made toward emphasis on alcoholism as a progressive, addictive disease for which total abstinence was the only remedy (reviewed in ref. *31*). In 1785, Benjamin Rush (reviewed in ref. *32*) described alcoholism as an addictive disease associated with toxic consequences of alcohol.

Linked with the disease concept was another concept that re-appeared in teachings by the Temperance Movement in the 19th century and by Alcoholics Anonymous and Jellinek in the 20th century: total abstinence was needed once a drinker became helpless with the appetite for alcohol. Although the requirement for abstinence by vulnerable individuals acquired many proponents for more than a century, optimism about moderate, controlled drinking seems prevalent among the majority of the population that has not experienced alcohol dependency. However, it may not be useful for individuals in the majority to rely on their own personal experience to balance the benefits and risks of moderate alcohol intake for individuals among the 5 to 10% who are vulnerable.

The concept of collectivity might be regarded an external result (phenotype) of multiple gene–environment interactions that are characteristic of the general human genotype. In that sense, different populations around the world that have open access to alcohol might have roughly similar aggregate alcohol intakes, with about 5 to 10% of people being vulnerable to heavy drinking. Such an approximate general outcome might occur from collective interactions of the heterogeneous mixtures of alleles among the diverse individuals of the population. For example, the prevalence, incidence, and lifetime risk of schizophrenia has been remarkably consistent across various populations and geographic areas during the past century *(33)*.

A broad look at mean alcohol consumption for people in different countries and cultural periods shows more similarity than might be expected. For example, Batel *(34)* noted that aggregate average annual per capita intake of alcohol in France is now near 3 gallons, and the prevalence of alcohol dependence may be about 6%. Average alcohol intake by the US population in 1994 was about 2.2 gallons, and the prevalence of alcohol abuse and dependence was about 7.4% *(35)*. These values are similar to those in other developed countries for which alcohol is second only to tobacco as a risk factor for disability-adjusted life years (DALYs; ref *36*; *see* Section 5.). Additionally, the current average annual consumption per capita in the United States is about 2 to 2.5 gallons of ethanol *(17)*; this amount remained at similar levels in the face of major cultural and lifestyle changes that occurred in the time preceding the Civil War (1850–1870; 2.2 gallons) and during the Industrial Revolution, with its waves of immigration, the advent of the automobile, and electric power (1881–1910; 2.2 gallons). Then, after limited access to alcohol during two World Wars, Prohibition, and the Depression, per capita consumption remained at similar levels during widespread social change that accompanied a shift from trains, radio, and movies (1945–1950; 2.1 gallons) to air travel and television (1960–1969; 2.2 gallons), space exploration and changing roles for women (1970–1989; 2.6 gallons), and home computers and the Internet (1990–1994; 2.3 gallons). One might wonder if there is some aspect of human biology that creates a characteristic 5 to 10% risk of alcohol use disorders.

Clear evidence for the heritable nature of alcohol dependency (e.g., ref. *37*) prompted an extensive genomic search to identify genes that contribute to the risk of the alcohol dependency disease, which appears in about 5 to 10% of the population. The study initially found suggestive evidence for genes that increase susceptibility to alcoholism on chromosomes 1, 2, and 7 as well as the possibility of a gene that decreases the risk of susceptibility to alcoholism on chromosome 4 *(38)*. Subsequently, three phenotypical factors were developed to account for 68% of the total variance in in more than 9000 individuals from alcoholic and control families *(39)*. These novel phenotypes may more

accurately model the effects of influential genes and help detect genes that are involved in this complex disorder. The most promising regions of linkage were on chromosomes 1 and 15. Progress in identifying genes for disorders and traits with complex inheritance has been slower than experts initially expected *(40,41)*. Slow progress is likely when many genes are involved for each phenotype and the effect of any one gene is small. More attention to defining narrower phenotypes is needed to detect genetic links in complex behavioral diseases.

4. BALANCING NUTRIENTS AND TOXINS

4.1. A Useful Nutrient?

Although biochemistry and physiology began developing insight into the three principal uses of alcohol (medical, nutritional, and recreational) during the 19th century, attitudes about alcohol shifted widely, ranging from it being a useful nutrient to a harmful toxin. The balance between academic theory and clinical practice shifted as new information developed. Physiological theories changed rapidly in the middle of the century, with scientific reports postulating a nutritive value for alcohol, then regarding it as worthless for nourishment purposes, and still later promoting its ability to lower body temperature *(42)*. However, most physicians remained unchanged in their clinical use of alcohol, balancing their continued belief in prior practice with the changing contradictory theories. Research results were accepted in constructing medical theories regarding how alcohol works, but they were largely irrelevant in the balance of opinions that formulated therapeutic practice. It may be humbling for scientists to realize that no matter how much scientific research results may change theories of alcohol action, theories may not be the basis on which clinicians recommend alcohol use nor the basis on which the public uses alcohol. Alcohol remained a widely prescribed therapeutic agent through the end of the 19th century despite the uncertainty regarding its nutritive value. A low public awareness of that uncertainty contrasts with widespread speculation about alcohol's putative role in preventing heart attacks.

By the end of the 19th century, an intense polarization had developed in the balance of attitudes between nutrient and toxin status of alcohol. The balance of power shifted during an unusual human tragedy involving Mary Hunt, National Superintendent of the Scientific Department of the Woman's Christian Temperance Union (WCTU), and Wilbur Atwater, first head of the United States Department of Agriculture (USDA) Experimental Stations *(32)*. Publishers of public school textbooks were under intense political pressure (led by Hunt) to include information on "hygienic physiology," which presented the viewpoint of the WCTU movement. The WCTU characterized alcohol as a dangerous and seductive poison of no useful value that could create an addictive appetite apt to become an uncontrollable disease. Alcohol addicts were regarded to have lost control and required complete abstinence from alcohol, renewing the concepts proposed by Rush a century earlier and preceding the positions by Jellinek and Alcoholics Anonymous in the 20th century *(31)*. For a generation of school children, these concepts were presented in textbooks as proven scientific findings.

However, by 1895, the political struggle for a new balance in power over alcohol physiology moved to include Atwater's unique calorimetric research on human alcohol metabolism *(43)*. Atwater campaigned vigorously that the public should regard alcohol as a valuable nutrient (although he agreed that it was not a food), and he attempted to reduce

negative teaching about alcohol in the public schools. He reported that alcohol was almost fully oxidized and replaced the caloric equivalent of either fat or carbohydrate (i.e., it acted as a food) *(44)*. Although Atwater (in concert with academic physiologists) succeeded in removing the polarized teaching about alcohol's toxicity from the schools, the political struggle led the Secretary of Agriculture to remove support for further alcohol experiments or for publishing the results in USDA bulletins. Eventually, limited support from the Carnegie Institution of Washington and the National Academy of Sciences assisted in distributing the experimental research results (*43*; also reviewed in ref. *32*). Atwater was joined by a "Committee of Fifty" academic physiologists, which claimed to focus on scientific studies of physical and social facts "without reference to the conclusions to which they might lead" *(45)*. The committee was an expression of the 19th century Liberal Movement. It attempted to integrate concepts of rational reform with four subcommittees: Physiological Aspects, Legislative Aspects, Economic Aspects, and Substitutes for the Saloons. Ironically, events combined to create a paradox in which, insisting on their lack of knowledge (and needs for more studies), the academicians "eliminated physiology from school curricula, and who, in the course of asserting their expertise over alcohol, made themselves irrelevant to the one American constitutional question (i.e., Prohibition) with a substantial physiological component" *(32)*. The intense public struggle between Hunt and Atwater came to an abrupt end in 1904 when Hunt lost power with the WCTU (and died in 1906) and Atwater was incapacitated by a series of possible strokes (and died in 1907).

The harsh political conditions may have diminished support for alcohol research for many years, and the advent of Prohibition in 1918 removed much motivation for academics to pursue scientific curiosity further. In 1935, the absence of organized continuing research led Mitchell to note *(46)* that the physiological value of alcohol was in a state of "utmost confusion," and World et al. *(6)* later suggested that alcohol-derived calories should possibly be disregarded completely as an energy source in predicting dietary-induced changes in weight. Later results from 89,538 women in the Nurses' Health Study and 48,493 men in the Health Professionals Follow-up Study showed that alcohol intake did not appear to contribute to overall body mass in women or men *(47)*. In fact, women had a clear inverse relationship between total energy intake of alcohol consumption and BMI.

This counterintuitive situation was supported by other studies *(48)* and by cumulative evidence from 31 separate surveys, which showed that reduced alcohol consumption probably would not help achieve or maintain a lower body weight *(49)*. Amazingly, the balance of information about alcohol as a nutrient remains uncertain *(50,51)*, with the nutritive value of alcohol calories still the subject of much debate and proposed research. Somehow, all of the scientific effort still has not provided a convincing resolution of the uncertainty. The debate at the Sixth European Congress on Obesity by Schutz *(52)* and Westererp *(53)* helped the scientific community become familiar with a topic that has remained unsettled for over a century (reviewed in refs. *54* and *55*). It seems likely that the issue of whether alcohol is a useful nutrient whose calories count the same as other calories will remain uncertain and controversial among scientists for some time.

Although since Atwater's study *(43),* calorimetric results have been believed to indicate that the energy of alcohol is handled similarly to that of other macronutrients, caloric compensation (in which one nutrient satiates appetite for other nutrients)

does not seem to occur with alcohol *(55–61)*. For example, men reported no decreased intake of average food energy (7555 kJ/d) or amounts of various macronutrients irrespective of an alcohol-altered average total energy intake, which ranged from 7576 to 9822 kJ/d *(47)*. Similarly, Rose et al. *(62)* reported that alcohol drinkers added alcohol energy to their diet rather than displacing food with alcohol, and there was no increase in BMI for women despite increased total energy intake with increased alcohol consumption. Alternatively, recent USDA results (obtained nearly 100 yr after Atwater's study) described alcohol being metabolized similarly to other foods *(63)*. Clearly, epidemiological surveys seem to contradict laboratory calorimetric studies, and one of the methods will eventually prove to give a correct interpretation. A hundred years of research still has not provided concurrence on alcohol's value as a nutrient, and serious questions about the consequences of alcohol intake perturbing metabolism and fuel selection require explanation.

4.2. Moderate Doses of a Toxic Substance?

The rationale of assigning the controversy over alcohol's toxic status to scientific examination by university professors diminished the flow of temperance-oriented education on alcohol as a toxin but did not provide alternative explanations, because researchers lost interest in the questions *(32)*. The balance of information on alcohol as a toxin shifted over the years with attention to the dose and to conditional toxic states in which foods and nutrients balance toxic actions of alcohol. When arguing for maintenance or repeal of Prohibition, different scientific arguments were used on both sides of the debate regarding intoxicating effects of alcohol *(64)*. Experimental psychologist Walter Miles measured impaired performance and urged maximizing human efficiency. In contrast, Carlson used low doses for which low signal-to-noise ratios prevented statistical proof of any alcohol effect. Support for ending Prohibition came from the chemical physiologist Yarnell Henderson, who focused on minimum performance necessary for the smooth operation of society. This view of "moderation" prevailed in the end, partly because its view of intoxication and the degree of hazard it represented was consistent with the personal experience of a majority of Americans (rather than the much smaller proportion that suffers from alcohol use disorders). Ironically, current maximum accepted exposures of toxicants are appreciably below the levels that were accepted when repeal was achieved in 1933. Modern scientists urge policies that minimize as much exposure to any known deleterious substance as possible *(64)*.

Efforts to interpret actions of alcohol have been confounded for decades by controversy over whether alcohol is toxic *per se* or whether it has a conditional effect that depends on interaction with foods and nutrients. Academic arguments continue over whether or not the diet during a given study was adequate in nutrients (such as choline, methionine, folic acid, thiamine, zinc, antioxidant vitamins, or carbohydrate) to prevent toxicity *(65)*. For example, animal models showed more alcohol toxicity with high-fat, low-carbohydrate diets *(66–68)*, and molecular mechanisms for impaired growth and liver pathology with alcohol remain the subject of controversy (*see* ref. *69*, Symposium on Nutritional Factors and Oxidative Stress in Experimental Alcoholic Liver Disease). Unfortunately, the development of documented mechanisms by which alcohol and its metabolite, acetate, perturb normal carbohydrate and fat metabolism has been slow *(28)*. Alternatively, progress in recognizing the complex network of cellular mediators and oxidant stress during alcohol-induced liver injury *(70)* was recently extended by detection of significant isoprostane

formation (a marker of tissue oxidant stress) during conditions similar to social drinking *(71)*. Results with such biomarkers are alerting the biomedical community to previously unsuspected alcohol-induced peroxidation *(72)* during intakes that many people regard as benign and "moderate." The paradoxical aspects of evidence for alcohol's possible antioxidant and pro-oxidant health benefits now border on the ironic.

For example, red wine has been central in many discussions about the possible cardiovascular benefits from alcoholic beverages *(73)*, particularly regarding preventing inflammatory vascular plaque formation. The published reports form an ambivalent balance in attributing the apparent success to ethyl alcohol (whether in wine, beer, or spirits) or to polyphenol antioxidants. The possible antioxidant suppression of transient oxidant stress during postprandial lipemia by red wine but not vodka *(74)* seems contradicted by the fact that endothelium-based, flow-mediated dilation is positively associated with non-wine alcohol consumption and negatively associated with smoking *(75)*. In contrast, polyphenols in de-alcoholized red wine reduced in vivo lipid peroxidation (as measured by isoprostanes in smoking subjects) *(76)*. A randomized controlled trial that reduced alcohol intake from 72 to 8 g/d showed no effect on endothelial-based, flow-mediated dilation *(77)*. However, median C-reactive protein (CRP) levels (possibly linked to inflammatory events) of 2.60, 2.20, 1.70, 1.60, and 1.80 mg/L were associated with alcohol intakes of less than one drink per month, one to three drinks per month, one to four drinks per week, five to seven drinks per week, and two or more drinks daily, respectively *(78)*. The decreasing CRP levels likely result from some lifestyle feature other than blood alcohol, and oxidant stress with more than one drink daily may raise CRP levels. The contradictory clues and the known risks of alcohol-related problems create uncertainty about the nature of possible benefits from alcoholic beverages.

5. BALANCING RISK FACTORS AND CAUSES

5.1. Death and Disease Burdens

Death is generally accepted as a precise condition, although the complexity of life makes it inevitable that there are a near infinite number of ways in which the complex adaptive living system arrives at death. Nevertheless, despite the multiple factors involved, each fatality is conventionally characterized by a single "cause of death." Statistical epidemiology associates factors with the listed cause of death. The association is predictive but is not proof of a causal role. Interpretations about associated risk factors need to be made with caution. For example, the risk factor most powerfully associated with all deaths is age, and tables of vital statistics are careful to stratify information by age groups to permit more meaningful age-adjusted interpretations. Nevertheless, age itself usually is not the direct cause of death, and health experts examine other causes when they design appropriate public health interventions. In the same sense, gender and socioeconomic status are important risk factors that are strongly associated with health outcomes for an individual, but they are not readily modifiable for intervention studies. Knowing mechanisms in the causal chain of disease events gives targets for successful intervention and ensures the likelihood of a credible solution. Epidemiologists must carefully examine the balance among risk factors and find more direct causal factors for which interventions can be arranged.

Social values and preferences of different populations require careful and prominent inclusion when discussing health problems and priorities linked to a possibly successful

intervention. To facilitate this inclusion, one recent study developed a standard unit known as the DALY to aid comparisons in a standardized approach to epidemiological assessment (ref. *79*, as reviewed in ref. *36*), and DALYs were estimated for each age–sex group to designate leading risk factors for death and disability in each major socioeconomic region. In this analysis, alcohol was a major risk factor, tied with unsafe sex as the third-ranked worldwide risk factor (3.5%) behind malnutrition (15.9%) and poor water, sanitation, and hygiene (6.8%). In established market economies, alcohol was second only to tobacco as a risk factor for DALY. In these economies, a possible protective effect of alcohol may have averted as many deaths as it caused (via its harmful effects), but worldwide alcohol caused 750,000 more deaths than it averted *(36)*. In 2000, exposure to alcohol caused 3.2% of the global deaths and 4.0% of the global DALYs *(80)*. Although age is strongly associated with mortality, it also has important effects on alcohol as a risk factor at different stages of life.

5.1.1. Pregnancy

Relatively low levels of alcohol disturb cellular signals in the developing fetus, causing serious lifelong physical and mental abnormalities for the individual; this finding led to national requirements for warning labels on alcoholic beverages. Late 19th-century reports showed that offspring of dogs who were given alcohol died young or were deformed, and inebriated inmates had more miscarriages and defective children than nonalcoholic inmates *(81)*. Researchers then demonstrated that alcohol impaired rat pup viability and guinea pig fertility and viability. Despite evidence of alcohol-impaired reproduction, experts asserted a lack of evidence that alcohol causes any abnormality in offspring *(81)*. Research on this topic was neglected for decades, until Jones *(82)* described Fetal Alcohol Syndrome, and the newly formed National Institute on Alcohol Abuse and Alcoholism shifted the balance of interest toward investigation of this important problem. Currently, the scientific community knows of fetal alcohol risks, but the information still is not uniformly transmitted or perceived by the public in ways that sufficiently alter behavior.

5.1.2. Infants and Children

Nursing infants had less active sleep during the 3.5 h immediately after exposure to alcohol in mothers' milk. Compensatory increases in active sleep were then observed in the next 20.5 h, when mothers refrained from drinking alcohol *(83)*. Infants also consumed less milk during the 4 h immediately after exposure to alcohol in mothers' milk *(84)*. Additionally, children in a household where one or both parents admitted drinking alcohol to change their state of mind or reduce dysphoric feelings (i.e., to escape) were more likely to dislike the odor of alcohol compared to children whose parents did not drink to escape *(85)*. Perceptions and priorities for alcohol are affected by early environmental interactions *(86)*.

5.1.3. Adolescents and Young Adults

Early access to alcohol is strongly associated with the eventual acquisition of alcohol dependence *(16)*, which has associated high social costs. In this regard, age restrictions on access to alcoholic beverages have been regarded as a useful preventive nutrition strategy. The very high DALYs and loss of life in traffic accidents (ranked ninth as a cause of worldwide DALYs) led to adoption by the United States of a nationwide, uniform, legal 21-yr age limit to restrict access to alcohol. In West Germany, Denmark,

Italy, and East Germany, traffic accidents involving alcohol and young men are the largest contributor to alcohol-related deaths *(87)*. Approximately 30% of deaths among males ages 25 to 44 yr are to the result of alcohol consumption.

5.1.4. Older Adults

Changes in personal priorities during life seem evident in the finding that 22% of heavy drinkers who previously met the Diagnostic and Statistical Manual of Mental Disorders IV criteria for alcohol dependence subsequently achieved total abstinence *(88)*, and an additional 50% reduced their consumption to a level matching that of persons who had never been classified as dependent. Most reduction occurred in individuals not participating in any type of alcohol treatment program, suggesting that individual current heavy drinkers may eventually voluntarily curtail their drinking. In this case, an eventual reduction in the proportion of heavy drinkers in the population might be achieved by continued prevention of new individuals from becoming heavy drinkers. Unfortunately, the worldwide history of collective attempts to diminish heavy alcohol drinking provides few encouraging examples of successful long-term tactics. Social support for total abstinence is an approach long-espoused by the 12-step facilitation therapy used in Alcoholics Anonymous. A recent large-scale clinical trial comparing outcomes from well-controlled therapeutic interventions showed sustained, modest success rates with matching treatment modality to different subtypes of patients, but it provided little indication of better successes than had been obtained with the 12-step facilitation therapy *(89)*.

5.2. Disease Risks Associated With Alcohol Use

An important confounding factor in interpreting results from epidemiological studies is the clustering of variables in patterns that reflect linked voluntary "lifestyle" choices, which prevents the variables from being treated as independent variables. This phenomenon was evident in the recent report on health professionals noted earlier *(7)*. With the commonality of linked lifestyle choices, one might ask the question: "When (and by what mechanism) during the wide variations in tissue alcohol levels could people obtain the putative benefits or risks associated with and attributed to alcoholic beverages?" Instinctive questions regarding the reason that the associative phenomenon occurs need to identify some causal mechanism before the effect can truly be understood. In approaching such questions, more precision in defining the attributes that are used in the statistical comparisons can greatly aid the interpretations. Clustering all cancer deaths or all cardiovascular deaths together fails to handle the diverse processes involved, and it greatly interferes with attempts to discuss preventive nutritional interventions that might decrease the prevalence of selected subtypes of tumor or cardiovascular process. Hypotheses about benefits and risks of drinking alcoholic beverages need to be examined with more precision than current epidemiological information provides. The following sections provide examples that illustrate some current knowledge about risks and possible benefits.

5.2.1. Cirrhosis

Alcohol drinking is associated with a dose-related risk of liver cirrhosis that progressively increases to dramatically high rates with heavier drinking levels, with relatively little risk associated with moderate drinking *(90)*. Mechanisms by which alcohol

can cause liver injury have been studied extensively, possible therapeutic targets have been suggested *(70)*, and recent evidence of oxidant stress at moderate drinking levels *(71)* points to a need for more detailed examination of underlying mechanisms *(91)*.

5.2.2. Cancer

Alcohol consumption has a clear association with cancer of the oral cavity, pharynx, larynx, esophagus, and liver and a suggestive association with cancer of the large bowel and breast *(92)*. Mortality from breast cancer is 30% higher among women who report consuming at least one drink daily compared to nondrinkers *(93)*. The finding that "moderate" intake of beer or spirits, but not of wine, was associated with considerable risk of cancer among Danes *(94)* might reflect an effect of different components of the beverages (rather than alcohol *per se*), or it might reflect associated lifestyle events that affected the risk of cancer. The similar finding that a lower risk for stroke existed among Danes who drank wine but not beer or spirits *(95)* seems provocative regarding possible lifestyle interactions. In contrast, a progressively increased relative risk for breast cancer was described for a different set of people *(90)*. With 3% of all cancers attributed to alcohol consumption *(96)*, there is a need to identify mechanisms whereby alcohol acts to increase or decrease the risk. One mechanism explored recently is that alcohol may increase risk of breast cancer by elevating plasma estrogen levels *(97)* in two ways: (a) through decreased clearance of plasma estrogen *(98)*, and (b) through increased absorption during hormone replacement therapy *(99)*. If these mechanisms cause increased breast cancers, then a prevention program aimed at women who are at high risk for breast cancer must be weighed against the possible cardiovascular benefits for moderate drinkers.

5.2.3. Hypertension, Stroke, and Heart Attacks

Reducing these major causes of death in the United States motivates a careful examination of all possible alternatives. Of 48 centers worldwide in which some people reported consuming at least 300 mL of alcohol per week, 35 centers had positive regression coefficients that linked heavy alcohol consumption to blood pressure *(100)*. Overall, alcohol consumption is associated with increased blood pressure, especially at the highest intake. The significant association of heavy drinking (three to four or more drinks per day) to blood pressure that was observed in both men and women and in younger and older men was independent of and added to the effect of BMI and urinary excretion of sodium and potassium on blood pressure. Therefore, hypertension appears to have many alternative causal factors as targets for preventive nutrition tactics, one of which is to decrease alcohol intake for some individuals. Different factors may be pivotal for different individuals, and each patient with hypertension must discern which factor is causal in his or her life.

Chronic alcohol intakes also appear to be linked with cardiac arrhythmias and atrial fibrillation in sudden cardiac death *(101)*, although no mechanism is known. Alternatively, among 22,071 men in the Physicians' Health Study, using those who consumed less than 1 alcoholic drink per week for comparison, the relative risks of all-cause mortality were 0.72, 0.79, 0.98, and 1.51 for those who consumed 2 to 4, 5 to 6, 7, and 14 or more drinks per week, respectively *(102)*. Similarly, a large cancer prevention study produced a 30 to 40% lower CVD death rate associated with moderate drinking *(93)*. Additionally, a population-based case–control study concluded that the relative risk of

a major coronary event was lowest among men who report one to four drinks daily on 5 or 6 d/wk and among women who report one or two drinks daily on 5 or 6 d/wk *(103)*. However, a much more cautious position was provided by Svarsudd *(104)*, who noted concerns over selection bias and limits in transferring conclusions from small subgroups to the general population.

Interventions for targeted individuals or situations in the form of therapeutic steps for clearly demonstrated pathology probably will have more overall support than general preventive interventions, for which the small minority that is at risk is unidentified and uncertain. In this case, individuals among the majority not at risk can easily lose confidence in the credibility of using preventive tactics that succeed only for a minority. Credibility is further strained when the mechanism of the disease or the intervention is not clear or has multiple alternatives—a situation encountered in balancing cardiovascular death and alcohol drinking. Interpreting cardiovascular death must overcome differences in understanding about whether blood cholesterol level is only a predictive risk marker associated with dietary and metabolic imbalance or whether it has a causal role in mediating the disease processes (*4,105*; *see also* Section 6.).

The certainty of diminishing thrombosis with low-dose aspirin has no counterpart for low-dose alcohol. Preventive nutrition approaches to decreasing cardiovascular mortality have long been regarded to involve reducing calories as total fat and saturated fat *(4)*, and molecular mediators of thrombosis, arrhythmia, and atherogenesis have been identified *(106)*. In fact, decreasing the formation and function of the n-6 eicosanoid thromboxane with low-dose aspirin inhibition of platelet cyclooxygenase is a strikingly effective tactic. This known mechanism has a parallel nutritional intervention in elevating the ratio of dietary n-3:n-6 essential fatty acids. Mechanisms by which alcohol might diminish fatal cardiovascular events remain unknown.

In considering the often discussed "French paradox" of low cardiovascular death rates relative to average blood cholesterol values, it is instructive to know that for subgroups of men ages 40 to 44 yr in 1960, 34% in France had died before reaching the ages of 70 to 74 yr, compared with 37% in the United States and 36% in England and Wales *(107)*. Although these overall rates appear similar, there was a dramatic difference in death rates resulting from ischemic heart disease (IHD): 4.5, 14.1, and 15.2%, respectively. However, if all men who died early of alcohol-associated causes had died of IHD, the rates would have been 21, 26, and 25%, respectively. Although the small unexplained residual difference in the three populations requires explanation, it is evident that the large portion of French men who die early from the serious negative impacts of alcohol tends to balance the low IHD death rate in the French population. That balanced "trade-off" is often ignored when discussing possible protection by moderate drinking. In 1990, estimates of 19 and 13% of all premature deaths in French men and women were attributed to alcohol *(108)*. Red wine consumption is not associated with reduced all-cause mortality in European countries, and, despite abundant observational data, proof that alcohol reduces CVD risk is weak in the absence of randomized controlled trials. Alcohol remains the third highest cause of preventable premature death in the United States *(109)*, and the risk of alcohol habituation, abuse, and adverse effects must be considered in any patient counseling. More controlled information on causal mechanisms (such as patterns of alcohol intake and mechanisms of cardiovascular death) needs to be weighed when balancing benefits and risks for complex multivariant situations.

6. BALANCING IGNORANCE AND KNOWLEDGE

The history of handling the "totality" of evidence on alcohol-related physiology provides interesting, and sometimes perplexing, examples of waxing and waning attention to evidence that presumably reflects changing attitudes and priorities. Developing wide-scale recommendations and social policies involves much more than scientific information, although such information can make the decision process more rational *(110)*. The information needed to alter behavior often is more than a statement of risk, and the ways by which behavior-modifying information travels within society requires deeper analysis. For example, people initially believed that warning labels on beverage bottles would alter behavior, but a postpolicy analysis of the value of the labels showed little effect *(111)*. The labels seemed to increase public awareness without appearing to have a major influence on drinking behavior (reminiscent of the use of alcohol by physicians in the late 19th century). In contrast to uncertain results with labels, restricting access to alcohol by changing the minimum legal drinking age from 18 to 21 yr significantly decreased the incidence of alcohol-related automobile crashes *(112)*. Additionally, young people then continued to drink less through their early 20s.

Expectations about possible personal benefits of moderate drinking by the majority (which is not particularly vulnerable to alcohol use disorder) rests on a balance with information about personal risks of moderate drinking. A thorough analysis of the risks and protective effects of alcohol on the individual was recently provided at a state-of-the-art conference sponsored by the Swedish Medical Research Council *(113)*. The organizers noted that each community or professional group has to examine the scientific literature to make policies that fit its own perceptions and priorities. The assembled reports of the conference facilitate such an examination. One overview balancing the risks and benefits of alcohol consumption *(90)* concluded by noting that younger and elderly adults are now advised to drink less than other adults and that men below age 35 yr, premenopausal women, and older persons who are not in good health are not likely to benefit from light-to-moderate drinking. A similar view of risks and benefits over the life span *(114)* noted the limited portion of the population that is likely to achieve demonstrable benefits from alcohol consumption, concluding with a reminder that priorities other than health are likely to continue to motivate alcohol drinking.

With Atwater's calorimetry, moderate alcohol intake was presented as scientifically proven to have beneficial effects on health (similarly to some current attitudes regarding cardiovascular risks). Hindsight permits recognition of the struggle to balance highly inflexible attitudes over positions that are now regarded as common knowledge: many people can drink moderately, although alcohol does cause major problems for some people *(31,32)*. The current awareness of genetic diversity and a shift away from the "melting pot" concept of creating a uniform, homogeneous American society suggest that revisiting the struggle between the Liberal Movement and the Temperance Movement might avoid the overpolarized positions that created historic weaknesses and lack of credibility. Currently, society still has no sure way to identify and assist the 5 to 10% of the population that is vulnerable to alcohol use disorders, and health benefits of alcohol remain unproved. Hopefully, the chronic lack of a solution for this large problem will spur sharper attention to causal factors and mediators for the disorders, thereby allowing for selection of targets for successful preventive interventions. With no certain answers, individuals remain free to choose their own personal accommodation to a risk for vulnerability.

Two recent large-scale public education efforts at preventive nutrition illustrated the challenge of advising a diverse population about heterogeneous disorders for which mechanisms are mostly not known and for which a large minority that is at risk in the population cannot be identified prior to disease initiation. The first effort was analyzed in a lengthy analysis of the National High Blood Pressure Education Program. Taubes *(115)* noted a philosophical clash between the public health policy requirement to act and the requirement of good science for institutionalized skepticism. Limiting the flow of information about alternative explanations to advance a single public health option may decrease some distractions, but it intensifies the risk of impaired credibility. Although some factors for various aspects of hypertension are recognized, controversy continues over general interventions that seem to oversimplify a complex issue in which many different factors act in different individuals. The decades-long debate *(115,116)* about salt intake and population-wide blood pressure management involves situations that are instructive for people who are contemplating preventive nutrition with alcohol. The second effort, which was even more relevant to the issue of whether alcohol decreases cardiovascular deaths, was the National Cholesterol Education Program *(117)*. In this effort, the clinical measure of elevated blood cholesterol associated with increased risk became a target for intervention *(118)*, and considerable controversy continued *(105,119)* over whether the elevated level caused the disease or was merely an associated marker. Were physicians treating the cause of the disease or only a symptom of it? With multiple mechanisms involved, CVD, like hypertension, should not be oversimplified when designing credible interventions. Although some causal mechanisms for various aspects of cardiovascular mortality (inflammatory atherogenesis, myocardial arrhythmia, platelet thrombosis) are recognized and are targets for intervention *(106)*, there is still no accepted hypothesis or mechanism by which moderate drinking of alcohol decreases risk. However, decreasing dietary saturated fats *(4,120)* and increasing dietary n-3 fats *(106,121)* or using low-dose aspirin *(122)* are effective preventive alternatives for decreasing causes of CVD. The onset and progression of CVD is not caused by a deficiency of alcohol intake that needs to be corrected. Rather, the chronic nutritional imbalances that exacerbate inflammatory atherogenesis, myocardial arrhythmia, and platelet thrombosis are rational targets for primary and secondary prevention *(106,123)*.

7. RECOMMENDATIONS

Exercising informed choice about foods from the wide range of acceptable options requires careful assessment of the context within which information about them was obtained and will be applied. Because a risk/benefit ratio for using alcohol in a preventive nutrition tactic involves balancing risks for developing one or more diseases against risks for developing alcohol use disorders, the following points should be considered.

A majority of individuals do not seem to be at risk for alcohol use disorders, but current insight into human genetic diversity allows recognition and acceptance that the effect of alcohol differs greatly among individuals. The risk from increased average drinking of alcohol involves a vulnerability to consume high levels that is not uniformly distributed among individuals. Generalizations from population medians or means do not adequately describe individuals in the vulnerable minority.

The median intake of alcohol in the United States may be about one to two drinks per week; this is much less than the proposed preventive level of one to two drinks per day,

which is currently consumed by only a small fraction of the population. Such intakes give very little tissue alcohol for most of the day.

People not yet exposed to alcohol (which includes every young person growing toward adulthood) are unable to predict whether or not they are among the 5 to 10% who are at risk for developing alcohol use disorders. Therefore, their election to drink can never be a fully informed choice between benefits and risks. The best guess about risk comes from an imprecise extrapolation from a family history of alcohol use disorder. As a result, majority acceptance of a restrictive alcohol policy for young people seems likely to continue as a primary prevention policy because of concern for the unidentifiable vulnerable minority.

Once genetic vulnerability collides with environmental facilitation, efforts at primary prevention end for that individual, and attention shifts to treatment and secondary prevention, for which total abstinence remains the principal successful option.

Risks associated with high levels of drinking are clearly evident for cancer, cirrhosis, and hypertension. However, the variance in those risks is wide enough for the results at low levels of alcohol consumption to permit many hypotheses to apply, including the null hypothesis. As a result, uncertain logic could alter the balance between designating alcohol as harmful or harmless, because what appears "true" for the complete general set may not be true for a specific subset.

Information on an observed associative relationship is suitable for hypothesizing about possible causes, but interventions with positive and negative controls are needed to prove a causal mechanism. Lifestyle choices of multiple variables confound associative relationships, decreasing the likelihood of assigning any one variable as the apparent cause. Currently, a causal role for alcohol in decreasing cardiovascular mortality has not been proved by controlled intervention studies.

Fully informed choice regarding the use of moderate drinking to prevent CVD requires weighing evidence of possible benefits and risks, much of which is currently indirect and imprecise. Health benefits of alcohol comsumption have been hypothesized from associative data, but no well-defined hypothetical mechanism is generally accepted for alcohol's actions, and no results from a well-controlled intervention have proved that alcohol causes the apparent benefit. To lower cardiovascular risks, preventive nutrition tactics other than alcohol consumption are available.

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34 Influence of Medication on Nutritional Status

Joseph I. Boullata

KEY POINTS

- Drug-induced changes to nutritional status may be considered a subclass of adverse drug effects.
- Medication may alter food intake, as well as cause weight loss or weight gain.
- Medication may alter macronutrient digestion, absorption, metabolism, and excretion.
- Medication may alter micronutrient absorption, distribution, metabolism, and excretion.
- Significant drug-induced alterations in nutritional status can be prevented or managed by selecting a therapeutic alternative without the unwanted nutritional effect or providing supplementation, in the case of nutrient losses.

1. INTRODUCTION

Preventive medicine includes nutritional approaches for promoting health and for preventing, delaying, or modifying disease processes. Avoiding poor nutritional status is one aspect of this that has wide ranging benefits to health outcomes. Recognition of risk factors for poor nutritional status is vital to this goal. Alterations in nutritional status may occur for a number of reasons, one of which is the result of drug–nutrient interactions. This chapter provides a perspective for clinicians on the potential changes in nutritional status resulting from medication use.

1.1. Medication Use

Use of medicines is a part of daily life for many people. It is estimated that about 80% of American adults use medication *(1)*. It should not come as a surprise that so many people use pharmaceutical products, whether prescription drugs, over-the-counter medicines, or natural health/dietary supplement products. However, aside from the promise of benefit, these products each have their own set of potential adverse effects resulting from the active ingredients and excipients—whether dose-related or not. Many of these adverse drug effects can impact on nutritional status. Interestingly, some of the well-recognized adverse effects of individual drugs occur as a direct result of

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alterations in nutritional status. So, in fact, drug-induced changes in nutritional status may be considered as a subclass of adverse drug effects. Given the enormous economic impact of drug use and the untoward consequences of this use, it is amazing that more attention is not regularly paid to their impact on nutritional status. Of the many issues influencing patient safety within the health care system that were catalogued in a recent national report, drug–nutrient interactions were not included among them *(2)*.

1.2. Interactions Between Drugs and Nutrients

There is a wide universe of potential drug–nutrient interactions that occur through various mechanisms, some of which can be clinically significant. A drug–nutrient interaction can be defined as the result of a physical, chemical, physiological, or pathophysiological relationship between a drug and nutrient, multiple nutrients, or food in general *(3)*. An interaction is considered significant from a clinical perspective if therapeutic response is altered or if nutritional status is compromised. Classification of the many interactions is often mechanistically based, with an identified precipitating factor and an object of the interaction, providing a management strategy for each (Table 1). Newly identified interactions can be categorized using this classification scheme. However, it seems that even the many known interactions are not well recognized by many health care providers or are only understood in more limited fashion (e.g., timing of drug administration with meals or specific foods). Based on a survey of health care providers, knowledge of a common drug–nutrient interaction was poor *(4)*. Few health care professionals even provide counseling about interactions to the majority of their patients *(5)*. Given the number of individuals with poor nutritional status, whether protein-calorie malnourished or obese or with specific deficits of individual nutrients (e.g., hypokalemia), each interaction may impact the potential safety and efficacy of commonly used medications.

The breadth of this topic is not only overlooked by some clinicians but also by regulatory agencies. This is particularly true in terms of identifying the nutritional consequences of each newly approved drug beyond simply the effect on appetite or body weight. Several less well-acknowledged alterations in nutritional status do exist. In fact, some side effects of medication may be the result of an imbalance of one or more nutrients. Such an effect, if clinically manifest, may be just as likely to be initially identified by nutrition specialists as a nutritional disorder as it would a drug side effect. The interference of drugs with nutritional status or status of a specific nutrient is often less readily evident clinically and more insidious in developing. Although not all drug-induced changes to nutritional status are overtly manifest (particularly with chronic medication use), even marginal deficits or tissue-specific deficits may have profound clinical effects *(6)*. Non-nutrient supplements (e.g., herbal medicines) have the same potential to alter nutritional status and nutrient disposition as other medicines *(7)*.

The impact of select drugs on nutritional status has been recognized for many years, particularly as synthetic drug development grew *(8,9)*. A few circumstances account for most clinically common drug-induced changes to nutritional status. These include drugs that cause significant gastrointestinal (GI) effects, including anorexia and malabsorption, or are nutrient antagonists by virtue of similar chemical structure and function as well as nutrients that are involved in multiple pathways. Anti-vitamin attributes have been ascribed to a number of drugs, yet even this may be the result of one or more different factors, including decreased absorption, reduced conversion to active form, interference with vitamin-dependent pathways, or increased vitamin clearance. An interaction is more likely with nutrients not stored

Table 1
Approach to Drug–Nutrient Interactions

Precipitating factor	*Object of interaction*	*Clinical management strategy*
Altered nutritional status	Drug	Aim to minimize treatment failure or drug toxicity
Food or food component	Drug	Aim to minimize treatment failure or drug toxicity
Specific nutrient	Drug	Aim to minimize treatment failure or drug toxicity
Drug	Nutritional status	Aim to maintain or improve nutritional status
Drug	Specific nutrient	Aim to maintain or improve status of individual nutrient

in great amounts (e.g., thiamin); those with complex absorption (e.g., vitamin B_{12}), distribution (e.g., zinc), and metabolism (e.g., vitamin B_6) characteristics; or those required for rapidly dividing tissues (e.g., folic acid, iron). Nutritional effects involving a few drug classes have been more frequently described. This is partly results from their chronic use in individual patients as a result of being in clinical use for decades or from their obvious impact on the GI tract. These drugs include antimicrobial agents (especially those used to manage tuberculosis), anticonvulsants, antineoplastics, and cardiovascular agents. Most other drug classes are likely implicated as well, but the drug approval process does not require studies to evaluate short- or long-term influences on nutritional status.

1.3. Clinical Implications

Adverse drug effects may not be attributed to a nutrient deficit but rather to the drug itself or the disease states being managed. An impact on the status of a nutrient may be clinically subtle, typically not resulting in a classic deficiency syndrome. Impact of medication in causing subclinical states of nutrient deficits is not as well appreciated as classic deficiencies. The lack of a wider recognition of drug-induced nutritional deficits may partly relate to the inadequate preparation of some clinicians for identifying malnutrition, especially poor micronutrient status. Therefore, sensitive and specific parameters for nutrient status need to be assessed regularly. In fact, alterations should be identified long before that degree of deficit is manifest, as the goal is to maintain or improve nutritional status. Ideally, every clinician should make an attempt to identify or predict drug–nutrient interactions to maximize patient outcome.

Other factors may increase the risk of drug-induced malnutrition. The clinical influence of a drug on nutritional status is not necessarily a fixed property of the drug itself but involves patient-related variables as well. The latter would include physiologically altered nutrient requirements, pharmacogenetic variability, a marginal diet, malabsorption, a chronic or catabolic disease, altered organ function, and concurrently ingested substances or environmental exposures. In other words, clinically significant outcomes of an interaction are patient-specific, not necessarily only drug-specific. All this presumes that nutritional status of patients receiving medication is regularly assessed in all health care settings *(10)*. A complete drug history is also required to identify all pharmacologically active substances administered—whether

prescription drugs, over-the-counter medicine, or dietary supplements. The latter group includes nutrient supplements, herbals and other botanicals, and non-nutrient/nonherbal supplements. A complete medical history and physical examination should be performed to identify diseases also associated with reduced nutritional status.

Recommendations to patients for improving or altering dietary patterns need to take into account any influence that this may have on medication use. Evidence-based nutritional recommendations should help prevent adverse drug reactions and maximize drug benefit. For example, this can include taking drugs appropriately with or without food, altering the drug dose in states of malnutrition, and avoiding or supplementing specific nutrients for specific medication regimens. Significant drug-induced alterations in nutritional status can be prevented or managed by selecting a therapeutic alternative without the unwanted nutritional effect or providing supplementation in the case of nutrient losses. However, data supporting the use of nutrient supplementation are still limited in most cases. Individuals using pharmaceutical products must ensure that their dietary habits and nutrient intakes reduce their risk of adverse drug effects.

This chapter is not intended to be exhaustive; additional lists of drug-induced nutrient depletions and more in-depth discussions are available *(11–13)*. Special mention needs to be made of examples that are not discussed further in the chapter. The many potential metabolic derangements (macro- and micronutrient) associated with the use of parenteral nutrition, a pharmaceutically complex prescription drug preparation after all, are well-recognized *(14)*. Ethanol is another drug responsible for significant alterations in nutritional status. Although this chapter does not address these, it should be remembered that use of ethanol increases the risk of the drug-induced nutritional deficits discussed in this chapter. Good reviews of the effect of alcohol consumption on nutritional status can be found elsewhere (*15,16*; *see* Chapter 33). Along these lines, it should be kept in mind that toxic ingestions (beyond the therapeutic use) of medications and related substances can also cause nutrient derangements. For example, acetaminophen overdose depletes glutathione and its rate-limiting amino acid cysteine, isopropyl alcohol ingestion causes hypoglycemia, and ethylene glycol causes hypocalcemia and depletes several B vitamins as well *(17)*.

2. MECHANISMS OF ALTERED NUTRITIONAL STATUS

Several mechanisms exist to explain drug-induced changes to nutritional status. These may involve influences on food intake, digestion, and absorption. Additional mechanisms involve influences on nutrient distribution, metabolism, and excretion. More than one mechanism can be in play for a given drug and nutrient. For example, the anti-epileptic agent carbamazepine decreases biotin status both by inhibiting intestinal absorption and accelerating metabolism to inactive catabolites *(18,19)*. A single nutrient may be influenced by more than one drug through a combination of mechanisms. For example, folate status may be affected by drugs that impair its absorption, alter protein binding in the circulation, block release from tissue sites, or enhance hepatic metabolism *(20)*. A single drug can have an influence on more than one nutrient through a single or multiple mechanisms. For example, by inducing a general malabsorption, colchicine increases loss of fat, nitrogen, sodium, and potassium in the feces with decreases in absorption of xylose, vitamin B_{12}, and, possibly, some carotenoids *(21)*. Corticosteroids, which are used in a wide variety of disorders, offer a further example. They have been noted to reduce levels of several nutrients (e.g., folate, vitamin B_{12}, selenium, and calcium) *(22,23)*. However, consideration needs to be given to the role played by the

pathophysiology that originally prompted the use of the drug (e.g., corticosteroids). This is not always evaluated in published reports, which makes it less clear whether the mechanism of altered nutritional status is exclusively drug-induced. Other factors, including poor dietary habits and lifestyle, may increase the risk of illness and subsequently require medication use; this topic is rarely discussed but is potentially relevant. The exact mechanism of altered nutritional status is not always fully understood. For example, the use of kava (*Piper methysticum*) may cause a yellowing of the skin that is unrelated to the hepatotoxic potential of this herbal medicine. It has been suggested that this may occur because of a niacin deficiency, although the mechanism remains unclear *(24)*. Of course, the influence of some drugs on nutritional status is the primary therapeutic action sought clinically (e.g., orlistat decreases fat absorption, pamidronate lowers serum calcium, warfarin reduces vitamin K availability).

Aside from appreciating the mechanism of an interaction, the clinical significance of many drug-induced alterations to nutritional status is described less frequently than most clinicians desire. This alone limits the ability to make recommendations, such as supplementation of the affected nutrient(s). Clinical judgment is needed in combination with available evidence to assess individual risk from an interaction.

The impact of drugs on nutritional status is not always predicted from animal studies and, as mentioned previously, is not routinely assessed during the drug approval process. Given the poor likelihood that each new drug will be evaluated for nutritional effects prior to marketing, clinicians should operate on the assumption that any variability in a patient's nutritional status is the result of a drug-induced change, unless proven otherwise. Manifestations typically are acute for a drug that interferes with a nutrient's metabolic activities, compared to interactions with nutrient intake, absorption, or clearance, which are expected to take longer to manifest. The biochemical, functional, or clinical manifestations depend on the degree of nutrient deficit (or excess) and the tissue compartment affected. Naturally, gender, age, and genetic factors all need to be considered. There is increased risk if the effects on nutritional status are additive and the result of chronic medication use or if the patient has marginal nutritional status to begin with. Risk to a patient from acute use of a single drug is expectedly less (especially in the face of adequate nutritional status) than that of an elderly patient with poor nutritional status requiring long-term use of one or more medications that impact on the status of a nutrient. Indeed, for many nutrients, subclinical deficits (i.e., nonclassic deficiency) may still be significant even when not recognized or identified.

There is no single best way to present information on drug-induced changes to nutritional status; for example, it could be described by drug class (If I take this drug what might happen?) or by nutrient class (If I have this deficit, what drug might have caused it?). Varying degrees of drug-induced changes in nutritional status are described in the following sections on food intake and digestion. This is followed by sections on the influence of drugs on macronutrient, vitamin, and mineral absorption, distribution, metabolism, and excretion.

3. FOOD INTAKE

A wide number of medications have the potential to alter food intake, thereby increasing the risk for poor nutritional status. Table 2 provides a list of select drugs that influence food intake. In addition to drugs with an obvious impact on appetite control, there are those that influence GI tract structure and function and some with an even less apparent role.

Table 2
Select Drugs That Alter Food Intake

Appetite stimulants
- Antihistamines
- Benzodiazepines
- Cannabinoids
- Clozapine
- Corticosteroids
- Insulin
- Lithium
- Mirtazapine
- Oral hypoglycemics
- Oxandrolone
- Phenothiazines
- Tricyclic antidepressants
- Valproic acid

Appetite suppressants
- Amphetamines
- Anticonvulsants
- Antineoplastics
- Atomexetine
- Bethionol
- Caffeine
- Dacarbazine
- Digoxin
- Epirubicin
- Etoposide
- Fenfluramine
- Fluoxetine
- Fluvoxamine
- Levodopa
- Methylphenidate
- Metronidazole
- Naloxone
- Nicotine
- Perhixiline
- Pimozide
- Sibutramine
- Temozalomide
- Thiazides
- Trazadone
- Zonisamide

Drowsiness
- Antidepressants
- Anti-emetics
- Antihistamines
- Antipsychotics
- Benzodiazepines
- Skeletal muscle relaxants

Depression
- Anticonvulsants
- Barbiturates
- Benzodiazepines
- β-blockers
- Clonidine
- Digoxin
- Levodopa
- Neuroleptics

Dry mouth
- Amantadine
- Antihistamines
- Antipsychotics
- Atropine
- Benzodiazepines
- Brompheniramine
- Captopril
- Cetirzine
- Clonidine
- Cyclopentolate
- Cyproheptadine
- Decongestants
- Didanosine
- Diuretics
- Flecainide
- Flunitrazepam
- Granisetron
- Isoniazid
- Mesalamine
- Molindone
- Nizatidine
- Ondansetron
- Oxybutynin
- Pentoxifylline
- Procainamide
- Propantheline
- Rimantadine
- Selegilene
- Sertraline
- Trazadone
- Tricyclic antidepressants
- Trimethobenzamide

Altered taste and smell
- Angiotensin-converting enzyme inhibitors
- Acetazolamide
- Albuterol
- Chlorpromazine
- Clarithromycin
- Clofibrate
- Metronidazole
- Nifedipine
- Opioid analgesics

(Continued)

Table 2 *(Continued)*

Allopurinol	Digoxin	Penicillamine
Amphotericin B	Diltiazem	Pentamidine
Antihistamines	Diuretics	Phenytoin
Aspirin	Ethambutol	Propranolol
Azathioprine	Flurazepam	Rifabutin
β-Lactam antibiotics	Gold	Quinidine
Baclofen	Griseofulvin	Selegilene
Bismuth salts	Iron	Sulfasalazine
Bromocriptine	Levodopa	Thioridazine
Carbamazepine	Lithium	
Chloral hydrate	Metformin	
Dysphagia		
Aldendronate		Iron
Anticholinergic drugs		Lidocaine (local)
Antineoplastics		NSAIDs
Antipsychotics		Neuromuscular blockers
Central nervous system depressants		Potassium
Corticosteroids		Quinidine
Immunosuppressants		Salicylates
Delayed gastric emptying		
Anticholinergics		Nitrates
Caffeine		Opiates
Calcium channel blockers		Oxybutynin
Clonidine		Theophylline
Dicyclomine		Tricyclic antidepressants
Iron		Verapamil
Meperidine		
Increased gastric emptying		
Bethanachol		Metoclopramide
Erythromycin		Misoprostol
Laxatives		

Drugs that alter vision or gait may limit the ability to gather food and prepare meals. Excessive hypotension and dizziness caused by medication may also restrict this ability. Cognitive disturbances caused by medication (e.g., psychotropics, clonidine, metoclopramide) also may lead to reduced food intake and subsequent weight loss. Consideration should even be given to drug-induced tremor as a cause for reducing food intake. Although unrelated to pharmacological effect, the impact (particularly for chronic medications) that out-of-pocket drug expense has on food choice compromise cannot be overlooked, especially in the elderly *(25)*.

Given the wide range of innervating neural pathways that affect the function of the GI tract, it is easy to appreciate that medication with pharmacological effects on cholinergic, histaminic, dopaminergic, opiate, serotonergic, or benzodiazepine receptors can influence GI function. Any drug that causes altered taste, dry mouth, oral pain, anorexia, nausea, vomiting, altered gastric emptying, diarrhea, or constipation can lead to impaired oral intake and nutrient absorption, which increases the risk for malnutrition.

Change in taste perception can have a significant influence on subsequent dietary intake. Medications can cause loss of taste (aguesia), distortion of taste (dysguesia), decreased

sense of taste (hypoguesia), and even gustatory hallucination (phantoguesia) *(13,26,27)*. Xerostomia as a result of inhibited saliva production (most commonly a result of drugs with anticholinergic properties) can also alter taste sensation. The list of drugs that cause dry mouth and altered taste sensation includes amitriptyline, bumetanide, cetirizine, didanosine, diphenhydramine, granisetron, imipramine, isoniazid, loratidine, mesalamine, nortriptyline, ondansetron, oxybutinin, pentoxifylline, selegiline, sertraline, trazodone, and trimethobenzamide *(13,27)*. Metered-dose inhalers that deliver medication allow some drug to precipitate in the oropharynx. This may also directly alter taste and reduce food intake. In the case of corticosteroid inhalers, the potential exists for fungal overgrowth in the region that also can reduce food intake. Oropharyngeal candidiasis may occur with chronic use of broad-spectrum antibiotics as well. If the causative agent cannot be discontinued or reduced in dose, then use of breath mints, lozenges, and sugarless gum may offer relief. Oral care regimens should be encouraged for those using metered-dose inhalers, and appropriate treatment should be implemented for candidiasis. If taste disturbances are the direct result of zinc deficiency, then supplementation of this mineral may benefit the individual *(26,28)*. Cytotoxic drugs can create inflammation of the oropharyngeal mucosal surface. This stomatitis severely curtails food intake because of the pain associated with eating. Additionally, several agents from this group of medications can cause a profound degree of nausea and vomiting.

Significant nausea may be centrally mediated (e.g., cytotoxic agents, opioid analgesics) or the result of local GI irritation. Vomiting may be a reported side effect of many medications; however, in most patients, continued intolerance to the medication only occurs with a select number of these drugs. Interference with nutritional status is observed with severe and prolonged emesis, as seen with cytotoxic chemotherapy. Poorly controlled nausea and vomiting significantly impact not only nutritional status but quality of life. Among the antineoplastic agents, some are much more emetogenic than others. The most likely to cause emesis—immediate or delayed—include aldesleukin, altretamine, carboplatin, carmustine, cisplatin, cyclophosphamide, dacarbazine, dactinomycin, daunorubicin, doxorubicin, epirubicin, idarubicin, ifosphamide, irinotecan, lomustine, mechlorethamine, mitoxantrone, pentostatin, and streptozocin *(29)*.

Drugs that increase GI motility are most likely to cause abdominal pain and diarrhea that, if severe or prolonged, will impair nutritional status by reducing intake, limiting absorption, and increasing nutrient losses. Drugs with direct effects on the GI tract mucosa include the nonsteroidal anti-inflammatory drugs and iron, whereas metoclopramide, erythromycin, and cisapride increase motility.

Again, although many drugs are known to cause diarrhea by one or more mechanisms, a recalcitrant few cause the most severe form. Diarrhea may be secretory or osmotic in nature; however, in either case, if diarrhea is severe, it will decrease dietary intake and create fluid, electrolyte, and other mineral losses. Drugs commonly associated with diarrhea include magnesium-containing antacids, antibiotics, antineoplastics, cholinergics (bethanechol, neostigmine), colchicine, digoxin, gold salts, mycophenolate mofetil, prostaglandins including misoprostol, quinidine, and the laxatives *(30)*. Some infrequently used antihypertensive agents (reserpine, guanethidine, methyldopa, guanabenz, guanadrel) can also cause diarrhea.

Constipation can be caused most commonly by agents that induce an ileus (e.g., opioid analgesics, anticholinergics, calcium or aluminum-containing antacids, barium sulfate, calcium channel blockers, clonidine, diuretics, ganglionic blockers, iron preparations,

nonsteroidal anti-inflammatory drugs, and polystyrene sodium sulfonate) *(30)*. Drugs with anticholinergic properties (including amitriptyline, atropine, belladonna, benztropine, diphenhydramine, hyoscyamine, imipramine, ipratropium, isopropamide, olanzapine, oxybutynin, procainamide, scopolamine, trihexyphenidyl, and zotepine) can reduce GI motility.

Weight loss has been associated with a number of medications beyond those that cause GI dysfunction. Decreased food intake can occur as a result of primary appetite suppression. Most of these are drugs that stimulate the nervous system centrally and peripherally and include the amphetamines, caffeine, methylphenidate, and theophylline. Intake may also be reduced by secondary effects of medication. Other agents that can produce an anorexic effect include the antihistamines, bethionol, dacarbazine, epirubicin, etoposide, fenfluramine, fluoxetine, fluvoxamine, levodopa, naloxone, nicotine, perhixiline, pimozide, sibutramine, temozalomide, trazodone, and zonisamide. Other causes of weight loss still need to be ruled out in patients using any of these medications.

Numerous drugs are associated with weight gain. This is most commonly reported with psychotropic medications, which may reinforce negative self-images held by some patients *(31)*. These drugs include amitriptyline, chlorpromazine, clozapine, imipramine, lithium, mirtazapine, and valproic acid *(32)*. Additional medications include insulin, oxandrolone and other steroids, and β-blockers. Drug-induced weight gain can be accompanied by the increased risk of morbidity associated with increased body weight from other causes. The weight gain from β-blockers results predominantly from accumulation of abdominal fat and may be more likely in younger patients and those with β-adrenergic receptor polymorphisms *(33)*. Weight gain induced by medication may be difficult to reverse, given that much of it is adipose tissue gain.

4. DIGESTION

The impact of medications on digestive processes is not well-documented. Drugs that are associated with pancreatitis or that cause damage to specialized cells of the GI tract can play a role in reducing digestion. Many drugs have been reported to cause pancreatitis, but the association is strongest for only a few. These include azathioprine/mercaptopurine, didanosine, the estrogens, ethanol, furosemide, metronidazole, pentamidine, the sulfonamides, thiazide diuretics, and valproic acid *(34)*. Maldigestion of fat is most likely to occur before impaired digestion and absorption of other macronutrients. The effect on digestion is expected to be most significant if chronic or recurrent bouts of pancreatitis are experienced. Any drug that creates a barrier between food particles and digestive components can also reduce digestion. Micronutrient malabsorption associated with changes in the digestive process includes the diminished availability of dietary vitamin B_{12} in the presence of chronic pharmacological acid suppression *(35,36)*. It can be expected that rapid intestinal transit induced by drugs that increase GI motility also increase the risk for maldigestion and subsequent malabsorption. This has the potential to influence both macro- and micronutrients.

5. MACRONUTRIENTS

5.1. Absorption and Distribution

Medication may cause malabsorption of carbohydrates, fats, and protein. Malabsorption of carbohydrates and fats is an expected therapeutic effect from the use of acarbose and orlistat, respectively. Both oral neomycin and colchicine are associated with carbohydrate

and fat malabsorption. Neomycin produces the classic example of dose-related drug-induced malabsorption *(37)*. By causing brush-border damage, an enteropathy arises, partly because of disaccharide intolerance and bile acid precipitation. Cytotoxic drugs (e.g., antineoplastics) can damage the absorptive surface of the GI mucosa. For example, methotrexate can cause macronutrient malabsorption following epithelial cell desquamation. The effects of antimicrobials (which alter the gut flora) on recovery of carbohydrates in the colon are not well-investigated.

The direct influence of drug use on the distribution or partitioning of monosaccharides, triglycerides and fatty acids, peptides, and amino acids between organs or tissues is not well-described. Lipodystrophy, including Fat Redistribution Syndrome, associated with the highly active antiretroviral therapy regimens used in patients with human immunodeficiecny virus (HIV) infection likely is multifactorial *(38)*.

5.2. Metabolism

Interference with metabolism of the macronutrients may result in altered circulating levels of glucose, cholesterol, triglycerides, or amino acids. Medications with a primary therapeutic effect of reducing blood glucose, triglycerides, or cholesterol are well-known and not mentioned further. In many cases, metabolic changes are transient but can be life-threatening.

Several drugs are associated with hyperglycemia or hypoglycemia (Table 3) *(39–41)*. Clinically, hyperglycemia is seen most commonly, caused by corticosteroids, β-blockers, thiazide diuretics, caffeine, calcium channel blockers, cyclosporine, growth hormone, morphine, nicotine, octreotide, phenytoin, protease inhibitors, sympathomimetics, tacrolimus, theophylline, and thyroid products *(39,41)*. The atypical antipsychotic agents (aripiprazole, clozapine, olanzapine, quetiapine, risperidone, and ziprasidone) have the potential to cause hyperglycemia and may increase the risk of diabetes *(42,43)*. The conventional antispychotics also may cause glucose intolerance, although they are more likely related to weight gain than to central glucose dysregulation *(43)*. As expected, hypoglycemia is most often associated with insulin and oral hypoglycemics; however, it is also associated with anabolic steroids, angiotensin-converting enzyme inhibitors, insulin-like growth factor (IGF)-1, octreotide, salicylates, tetracycline, and warfarin *(40,41)*.

Hyperlipemia is an adverse effect resulting from a number of drugs, including anabolic hormones, β-blockers, diuretics, progestins (including those in combination oral contraceptives), danazol, immunosuppressive agents, and protease inhibitors *(44)*.

Corticosteroids increase endogenous protein catabolism, most likely by increasing activity of the ubiquitin-proteosome proteolytic pathway *(45,46)*. Protein synthesis can be increased as a result of growth hormone, anabolic hormones, and IGF-1 without impacting on protein degradation *(47)*. Azotemia as a manifestation of drug-induced effect is more likely a result of impairing renal excretory function than interfering with protein metabolism. Very little information is available regarding the impact of medication on specific amino acid absorption, transport, or metabolism. Some significant adverse effects attributed to valproic acid (e.g., hepatotoxicity, teratogenicity) may involve drug-induced alteration in the methionine cycle *(48)*, and oral contraceptives may increase tryptophan metabolism *(49)*.

A drug that is used in the management of tardive dyskinesia was found clinically to cause severe lactic acidosis, hypoglycemia, hyperammonemia, and acute hepatic encephalopathy in patients. This set of adverse drug effects was eventually traced not to

Table 3
Drug-Induced Changes to Blood Glucose

Hyperglycemia	
Acetazolamide	Minoxidil
Atypical antipsychotics	Morphine
β2-Agonists	Nicotine
β-Blockers	Octreotide
Caffeine	Oral contraceptives
Calcium channel blockers	Pentamidine
Corticosteroids	Phenothiazines
Cyclosporine	Phenytoin
Cyproheptadine	Protease inhibitors
Dapsone	Rifampicin
Diazoxide	Sympathomimetics
Growth hormone	Tacrolimus
Isoniazid	Theophylline
Lithium	Thiazide diuretics
Loop Diuretics	Thyroid hormone
Hypoglycemia	
Angiotensin-converting enzyme inhibitors	Monoamine oxidase inhibitors
Acetaminophen	Mebendazole
β-Agonists	Nonselective β-blockers
Benzodiazepines	Octreotide
Biguanides	Pentamidine
Cotrimoxazole	Quinidine
Disopyramide	Quinine
Ethanol	Salicylates
Fluoxetine	Sertraline
Ganciclovir	Sulfonylureas
Gatifloxacin	Tetracycline
Insulin	Tricyclic antidepressants
Lithium	Warfarin

altered macronutrient disposition but to the induction of pantothenic acid deficiency by the drug *(50)*. This illustrates the complexity of drug-induced changes in nutrient status as well as the difficulty in identifying them.

5.3. Excretion

Drug-induced diarrhea or impairment of renal function (reabsorption or concentrating ability) leads to the unrecoverable loss of macronutrients, possibly with significant consequences.

6. VITAMINS

A number of drugs can influence vitamin status through effects on absorption, distribution, metabolism, and excretion. These drugs are discussed in the following sections, and a list of select changes in vitamin status can be found in Table 4.

Table 4
Drugs That Alter Vitamin Status

Vitamin A		
Cholestyramine	Mineral oil	
Ethanol	Neomycin	
Vitamin D		
Aluminum antacids	Isoniazid	Phenytoin
Bisphosphonates	Mineral oil	Rifampicin
Cholestyramine	Orlistat	Sunscreen
Cimetidine	Phenobarbital	
Vitamin E		
Aspirin	Gemfibrozil	Oral contraceptives
Cholestryramine	Loop diuretics	Orlistat
Corticosteroids	Mineral oil	
Vitamin K		
Aminoglycosides	Fluoroquinolones	Phenytoin
Barbiturates	Mineral oil	Sulfonamides
Cephalosporins	Oral contraceptives	Tetracyclines
Cholestyramine	Orlistat	
Thiamin		
Aminoglycosides	Fluoroquinolones	Sulfonamides
Cephalosporins	Loop diuretics	Tetracyclines
Digoxin	Oral contraceptives	Theophylline
Ethanol	Phenytoin	
Riboflavin		
Aminoglycosides	Doxorubicin	Quinacrine
Amitriptyline	Fluoroquinolones	Sulfonamides
Cephalosporins	Imipramine	Tetracyclines
Chlorpromazine	Oral contraceptives	
Niacin		
Aminoglycosides	Isoniazid	Valproic Acid
Cephalosporins	Sulfonamides	
Fluoroquinolones	Tetracyclines	
Pyridoxine		
Aminoglycosides	Ethanol	Oral contraceptives
Cephalosporins	Fluoroquinolones	Penicillamine
Cycloserine	Hydralazine	Sulfonamides
Dopamine	Isoniazid	Tetracyclines
Estrogen	Loop diuretics	Theophylline
Vitamin B_{12}		
Aminoglycosides	Fluoroquinolones	Phenytoin
Antivirals	H_2 antagonists	Proton pump inhibitors
Cephalosporins	Metformin	Sulfonamides
Cholestyramine	Neomycin	Tetracyclines
Colchicine	Oral contraceptives	Zidovudine
Folic Acid		
Aspirin	Metformin	Phenytoin
Carbamazepine	Methotrexate	Potassium-sparing diuretics
Celecoxib	NSAIDS	Primidone

(Continued)

Table 4 *(Continued)*

Cholestyramine	Olsalazine	Pyridoxine
Corticosteriods	Oral contraceptives	Sulfasalazine
H_2 antagonists	Pancreatic enzymes	Valproic acid
Indomethacin	Phenobarbital	
Choline		
Cyclosporine	Nifedipine	
Daunomycin	Verapamil	
Biotin		
Carbamazepine	Phenytoin	
Phenobarbital	Primidone	
Vitamin C		
Hydrocortisone	Oral contraceptives	Salicylates

6.1. Absorption

Hundreds of transporter genes have been identified in the human genome, many of which express transport proteins at the GI tract for vitamins, amino acids, peptides, and others; some of which may also be used by drugs *(51)*. This points to the potential for many drug–nutrient interactions that have not yet been investigated. The degree of influence that a drug may have on the absorption of a micronutrient may depend on meal composition.

6.1.1. Water-Soluble Vitamins

Nutrients with a carrier-mediated intestinal transport system pose a potential risk for an interaction with drugs using the same or associated transport mechanisms for absorption. For example, some P-glycoprotein substrates (e.g., cyclosporine, verapamil, nifedipine, daunomycin) may inhibit choline uptake directly or indirectly *(52)*. Additionally, intestinal absorption of biotin can be limited by competition with carbamazepine for the same transporter *(18)*.

Several drugs that use the folic acid transporters, include sulfasalazine, olsalazine, and methotrexate, have the potential to decrease absorption of folic acid *(53)*. Methotrexate competes with folic acid for absorption and contributes to folate deficiency in patients treated even with low doses of this drug *(54,55)*. The folate deficit can account for some of the toxicity of the drug, and repletion of folic acid during methotrexate therapy does not interfere with drug efficacy *(56,57)*. Triamterene has been reported to cause megaloblastosis, which may result from competitive inhibition of folic acid absorption *(58–60)*. However, folate depletion does not occur in all patients treated with this diuretic *(61)*. Pancreatic extracts can impair folic acid absorption as well *(62)*. Cholestyramine has also been reported to reduce bioavailability of folic acid *(63,64)*.

Many gut microorganisms, including several strains of *Bifidobacteria,* can synthesize B vitamins—particularly thiamin, niacin, and folate *(65,66)*—and can be a source of several vitamins *(67)*. Although the relative importance of bacterially derived B vitamins or vitamin K to human nutrition remains unclear, antimicrobials, which severely reduce the number of bacteria that produce these nutrients in the GI lumen, may impact individuals with borderline status of these vitamins.

Reversible vitamin B_{12} malabsorption has been described for colchicine—whether administered orally or parenterally *(21,68)*. It is not clear whether this represents a specific

inhibition of transport involving the intrinsic factor-vitamin B_{12} receptor or if it is a result of general mucosal damage described previously *(69,70)*. Serum vitamin B_{12} concentrations remain normal, although given the potentially large body stores of vitamin B_{12}, normality depends on the baseline status of this vitamin and duration of drug treatment *(71)*. Although vitamin B_{12} malabsorption is identified among individuals with advanced stages of HIV infection, subnormal serum concentrations of this vitamin may occur more frequently in those receiving zidovudine therapy *(72)*. Biguanides (e.g., metformin) can induce malabsorption of vitamin B_{12} in as many as 30% of patients using them *(73,74)*. Although this may be reversible in some patients, it is likely that about half of patients exhibit depressed secretion of intrinsic factor that may not be reversible *(73)*. Despite the fact that not all patients exhibit clinically symptomatic deficiency, it may be associated with increased total serum homocysteine levels *(74,75)*. Furthermore, hematological and especially neurological complications of vitamin B_{12} deficiency may not become evident for several years.

Histamine-type 2 (H_2) receptor antagonists and proton pump inhibitors (now also available without a prescription) significantly decrease dietary vitamin B_{12} absorption as a result of decreased cleavage from food in the presence of lower levels of gastric acid and pepsin *(35,36,76–78)*. H_2 receptor antagonists may even reduce intrinsic factor secretion *(79)*. This reduction is expected to become clinically evident following at least 1 to 2 yr of therapy, particularly in patients with inadequate intake. Adequate bioavailability from vitamin B_{12}-containing meals that contain an acidic beverage may account for variability of time to present with clinical deficiency (if present) *(35,80)*. As expected, this therapy does not interfere with the absorption of crystalline vitamin B_{12} preparations *(76)*. Consideration should be given to vitamin B_{12} supplementation in patients using these medications chronically. Closer examination is needed to determine whether drug-induced increases in gastric pH influence the absorption of other nutrients. For example, β-carotene absorption may be reduced at lower levels of gastric acidity *(81)*.

6.1.2. Lipid-Soluble Vitamins

Orally administered mineral oil can cause gross malabsorption of nutrients. Lipid-soluble nutrients, including vitamins A, D, E, and K as well as the carotenoids, solubilize in the oil and become trapped within the lumen. Given the rapid GI transit, digestion is incomplete and absorption is reduced, partly because of the rapid transit but also as a result of the mechanical barrier formed around food particles *(82)*. Probucol and cholestyramine treatment has been reported to significantly reduce serum β-carotene and lycopene concentrations *(83)*. By binding bile acids, cholestyramine reduces the absorption of the fat-soluble vitamins *(63,84)*. The potential for developing osteomalacia and bleeding disorders has been suggested *(85)*. One study suggested no significant effect on vitamin D status in patients using cholestyramine chronically, although bone density measurements were not performed, and vitamin D samples were obtained during spring and summer *(86)*.

By inhibiting lipase action, orlistat not only induces lipid malabsorption but also decreases the absorption of fat-soluble vitamins *(87)*. Vitamin E absorption may be reduced by over 50%, whereas vitamin A is not as significantly influenced *(88)*. Vitamin supplementation may be required for some patients. However, in a study in which patients received a lipid-soluble vitamin-containing supplement during treatment with orlistat, serum concentrations of both vitamins D and K were still reduced *(89)*. In fact,

the reduction in vitamin D despite supplementation warranted a recommendation for regular monitoring in patients receiving orlistat, even when supplementation is included as part of the regimen. The absorption of a pharmacological dose of β-carotene may also be reduced by about one-third during treatment with orlistat *(90)*.

Hepatic menaquinone (vitamin K) content may be reduced following broad spectrum antibiotic use *(91)*, but it remains to be determined whether this is a cause–effect relationship or if it is clinically significant. In fact, alternative explanations to vitamin K deficits have been offered for changes in blood clotting in patients receiving antibiotics, which includes cases of bleeding following cephalosporin use *(92)*.

6.2. Distribution

6.2.1. Water-Soluble Vitamins

Red blood cell folate levels are lower in patients treated with the anticonvulsants phenytoin and carbamazepine, but this is not correlated with dietary folate intake *(93)*. Phenytoin may actually reduce tissue uptake of folate *(94)*. Additionally, thiamin levels in the cerebrospinal fluid are lower in patients being treated with phenytoin compared to controls *(95)*. Uptake of thiamin and its phosphoesters may vary by nervous tissue region during phenytoin treatment *(96)*. Intracellular loss of thiamin from cardiac cells occurs in the presence of furosemide or digoxin, with reduced ability of the cells to take up the vitamin *(97)*.

Folate binding and serum folate may be reduced in patients who are using aspirin, with a possible rise in urinary folate excretion *(98,99)*. Aspirin also inhibits the uptake of ascorbic acid into tissues, including white blood cells. This can reduce tissue saturation, possibly leading to tissue depletion in patients chronically treated with aspirin and creating opportunity for greater renal excretion *(100–102)*. The saturable, high-affinity ascorbic acid transporter may be inhibited by hydrocortisone *(103)*.

Zidovudine can deplete carnitine in muscle, which is associated with drug-induced myopathy *(104)*. Tissue carnitine depletion during treatment with valproic acid may partly result from an inhibition of tissue uptake *(105)*.

6.2.2. Lipid-Soluble Vitamins

The mechanism that leads to decreased levels of α-tocopherol, γ-tocopherol, and ubiquinone-10 (co-enzyme Q10) that are described with gemfibrozil therapy remains unclear *(106)*. The dose-dependent reduction in tissue and circulating levels of ubiquinone-10 levels with hydroxy-methylglutaryl-co-enzyme A reductase inhibitors (e.g., lovastatin, pravastatin, simvastatin) likely result from inhibited synthesis *(107–109)*. The lower ubiquinone levels may contribute to impaired mitochondrial function, as determined by lactate-to-pyruvate ratio *(110)*. This may partly explain the drug-induced hepatic dysfunction seen with this class of agents. However, the decline may be the natural result of total cholesterol decline, which does not require further attention *(111)*. Supplementation with an appropriate co-enzyme Q-containing product may prevent the depletion, although the clinical value of this intervention has yet to be determined *(112)*.

6.3. Metabolism

6.3.1. Water-Soluble Vitamins

A number of drugs can interfere with vitamin metabolism. The metabolism of pyridoxine is impaired by several drugs, including isoniazid, cycloserine, and hydralazine.

The potential for a secondary deficiency of niacin also exists. Reduced circulating levels of pyridoxine and thiamin occur more frequently (and in a dose-dependent manner) in patients treated with theophylline than in similar patients not treated with this agent *(113–115)*. Plasma pyridoxal-5′-phosphate appears to be more affected than plasma pyridoxal or urinary 4-pyridoxic acid *(115)*. Theophylline is a pyridoxal kinase antagonist that, with chronic use, may require concurrent vitamin B_6 supplementation to limit nervous system side effects such as tremor *(116,117)*. This differs from the effects of dopamine, isoniazid, cycloserine, hydralazine, and penicillamine, which form covalent complexes with pyridoxal or pyridoxal-5′-phosphate and thereby inhibit pyridoxal kinase *(116)*. The poor availability of this vitamin, which reduces γ-aminobutyric acid levels in the brain, may play a role in the seizure risk of theophylline at high concentrations. Hydralazine-induced deficiency of vitamin B_6 has been reported to cause neuropathy *(118)*. Through its interaction, hydralazine appears to inhibit a number of enzymes that require pyridoxal as a cofactor, and this might contribute to the drug's hypotensive effect *(119)*.

Several drugs can impair the conversion of riboflavin to active co-enzymes. These include chlorpromazine, amitriptyline, imipramine, quinacrine, and doxorubicin *(120,121)*.

A large portion of patients treated with sulfasalazine develop folate deficiency *(122)*. The deficiency may be responsible for severe megaloblastic anemia and severe pancytopenia, which are associated with the drug *(123,124)*. Although malabsorption may play a partial role (as mentioned previously), metabolic abnormalities may also contribute to the deficit *(125)*. Several nonsteroidal anti-inflammatory drugs can also interfere with the impact of folate metabolism on biosynthesis of other nutrients *(125)*. Although not all patients treated with these agents exhibit a significant decrease in serum folate levels, tissue levels of folate may be reduced in the absence of serum folate abnormalities.

Early descriptions of the influence of oral contraceptives on nutritional status described decreased levels of several B vitamins and ascorbic acid, whereas levels of vitamin K increased *(126)*. Compared to nonusers, users of oral contraceptives exhibited lower levels of erythrocyte folate and riboflavin, erythrocyte transketolase activity, plasma pyridoxal phosphate, and serum vitamin B_{12} within a few cycles of initiating treatment *(127–129)*. Increases in urinary formiminoglutamic acid in women using oral contraceptives indicate alteration in folate metabolism *(128)*. Reduced folate status in these women needs to be addressed prior to discontinuing use of oral contraceptives in those of childbearing age. The value of folic acid supplementation in women using oral contraceptives may extend beyond prevention of low circulating levels to prevention or reduction of cervical dysplasia *(130,131)*. Oral contraceptives may increase tryptophan metabolism through induction of tryptophan oxygenase, and it has been theorized that this may lead to a pyridoxine deficiency *(49,129)*. The deficits have been associated with clinical symptoms, including depression and anxiety. In such cases of deficiency, vitamin B_6 repletion may require doses of at least 40 mg daily to correct levels and clinical manifestations *(132,133)*. Clinical manifestations are not universally responsive to vitamin supplementation at pharmacological doses *(134)*. Incomplete evaluation of baseline and follow-up status may explain the variability in findings with repletion. This was the case for riboflavin status in oral contraceptive users. Despite several reports of poor riboflavin status in users *(135,136)*, when diet is controlled, the need for supplementation vanishes *(137)*.

Oral contraceptive formulations may also have an influence on ascorbic acid status *(138–140)*. Reductions in plasma and leukocyte levels of this vitamin have been described, although the mechanism remains unclear. Oral contraceptive users appear to have a more rapid turnover of ascorbic acid *(141,142)*. Although increased utilization and metabolism is a possible mechannism for this rapid turnover, it may also be explained by alterations in tissue distribution *(139)*. It is possible that with adequate dietary intake, no significant change in ascorbic acid status occurs, and individuals using oral contraceptives would rarely be expected to require supplementation *(143,144)*.

Biotin catabolism can occur with chronic use of the anticonvulsants carbamazepine, phenytoin, phenobarbital, and primidone but not with valproic acid *(145–147)*. This can be associated with increased organic aciduria. The loss of biotin may contribute to the adverse effects of these anticonvulsants, including poor seizure control. Many patients have difficulty tolerating the side effects of carbamazepine, which may partly result from altered nutrient status. Biotin deficiency may be the most significant of the nutrient deficits (*see* folate and vitamin D discussions below) that are caused by the anticonvulsants *(148)*. Supplementation of biotin to help control seizure activity awaits clinical trials.

Serum folate levels were noted to be significantly lower in children treated for epilepsy with anticonvulsants (phenobarbital, phenytoin, primidone) *(149)*. Erythrocyte folate levels are also noted to be reduced in patients being treated with phenytoin and/or carbamazepine but not with valproic acid *(93)*. This was not correlated with dietary folate intake. Serum folate levels decrease following initiation of phenytoin therapy. Conversely, folic acid supplementation alters the pharmacokinetics of phenytoin. Because of the two-way interaction between this drug–nutrient pair, administration of folic acid supplementation at the initiation of phenytoin therapy prevents the decrease of folate and allows quicker achievement of steady-state phenytoin concentrations *(150)*. Negative studies may involve patients who have maintained adequate folate status since before initiation of anticonvulsants *(151)*. Enzyme induction may be involved in the reduction in folate status in patients treated with the anticonvulsants phenobarbital, phenytoin, or carbamazepine compared to controls *(152,153)*. The effects of carbamazepine may be more severe than the others and are likely dose-related, and supplementation with a folic acid analog may improve some cognitive measures in patients treated with the drug *(93,152)*. Disturbances of folate and thiamin status have been suggested as an explanation for adverse neuropsychiatric effects of anticonvulsants *(154,155)*. The effects from carbamazepine may take about 2 mo to manifest *(156,157)*. Outright megaloblastic anemia may be most likely in patients who have a poor folate status prior to treatment. Although reductions in folate status have been noted for phenobarbital, phenytoin, and carbamazepine, valproic acid treatment does not appear to disturb this vitamin *(93)*. In contrast, carnitine deficiency can occur with valproic acid treatment *(158,159)*. Reductions are noted in both plasma free carnitine and plasma total carnitine concentrations *(158,159)*. A reduction in urinary total and free carnitine has also been described for chronic valproic acid treatment *(160)*. Valproic acid can inhibit the hepatic synthesis of carnitine and contribute to a deficiency state. It appears to occur at the level of butyrobetaine hydroxylase but is without direct inhibition, likely a result of reduced α-ketoglutarate levels, which are required as a cofactor *(161)*. This deficit may contribute to the drug's adverse effects, including hyperammonemia. Management of clinical deficiency required a significant dose of carnitine in children, in which case symptoms resolved within 1 wk of the intervention *(159)*. An appropriate

prophylactic dose has not been described. Intravenous administration of L-carnitine is recommended for patients with valproic acid induced hepatotoxicity or other acute metabolic crises associated with carnitine deficits *(162)*. It is suggested that oral L-carnitine supplementation be considered for patients with symptomatic valproic acid-associated hyperammonemia or for those with multiple risk factors for valproic acid hepatotoxicity as well as infants and children using valproic acid *(162)*. The recommended oral dose of L-carnitine is 100 mg/kg daily to a maximum of 2 g daily. Supplementation may not be needed in patients receiving valproic acid who are otherwise healthy and ingest a regular diet *(163)*.

6.3.2. Lipid-Soluble Vitamins

Although not often mentioned, most topically applied sunscreen drug products form a barrier to ultraviolet light that can reduce vitamin D formation in the skin *(164)*. Osteomalacia resulting from altered nutrient availability has been attributed to a number of medications beyond sunscreens. These include cholestyramine, phenytoin, phenobarbital, isoniazid, aluminum antacids, bisphosphonates, fluoride, and rifampicin.

Isoniazid is noted to impair both hepatic and renal vitamin D metabolism *(165)*. However, the combination of isoniazid and rifampicin did not appear to alter vitamin D metabolism over a 9-mo treatment period *(166)*, despite a study in normal subjects in which a brief course of oral rifampicin decreased circulating 25-OH-cholecalciferol levels by more than 50% *(167)*.

The anticonvulsants phenobarbital, phenytoin, and carbamazepine can reduce vitamin D by altering its metabolism through enzyme induction, thereby reducing serum 25-OH-cholecalciferol *(168–172)*. Determining bone mineral densities may be useful in detecting bone loss, especially in children receiving anti-epileptic agents *(173)*; however, skeletal changes may be identified only by bone biopsy *(174)*. Patients treated with anti-epileptics may have reduced bone mass in the absence of obvious vitamin D deficiency *(175)*. Elevated alkaline phosphatase in patients who had low serum 25-hydroxyvitamin D [25(OH)D] while receiving carbamazepine normalized following vitamin D supplementation *(176)*. However, the use of vitamin D supplementation may not be warranted in all patients receiving carbamazepine *(177)*. Nevertheless, monitoring vitamin D status is justified. Interference with vitamin K metabolism may be a lesser factor in side effects of phenytoin and phenobarbital on bone *(178)*. Cimetidine inhibits 25-hydroxylase activity in the liver, thereby reducing vitamin D hydroxylation, but serum 25(OH)D rises within 1 mo of discontinuing therapy *(179,180)*. Effects of medication on vitamin D vary, which may partly result from the complexities of formation, activation, and metabolism, not to mention polymorphism of the vitamin D receptor.

It is unknown whether drugs that increase cytochrome P450 activity alter metabolism of vitamin E isomers or if they alter vitamin status, given the relevance of this enzyme system to initial steps in vitamin E metabolism *(181)*.

6.4. Excretion

There appears to be a depletion of folic acid and vitamin B_{12} with long-term use of metformin *(75)*. This depletion is also noted with long-term use of diuretics *(182)*. Diuretics may increase renal losses of thiamin that contribute to symptomatic heart failure, although these patients often have poor thiamin intake as well *(183)*. Patients with

congestive heart failure are likely to exhibit thiamin deficiency, which not only resolves with thiamin treatment but also improves left ventricular ejection fraction *(184,185)*.

Chlorpromazine and tricyclic antidepressants (e.g., amitriptyline) increase urinary excretion of riboflavin *(186,187)*. It is unclear whether this is the end result of decreased tissue distribution or the result of reduced metabolic incorporation. Flavin adenine dinucleotide production from riboflavin may be inhibited by these drugs, given the structural similarities *(188)*. Furthermore, tricyclic antidepressants may inhibit a number of riboflavin-dependent enzymes (e.g., nicotinamide adenine dinucleotide phosphate oxidase) involved in mitochondrial respiration in cardiac tissue. It has not been established whether this inhibition relates to the potential cardiotoxicity of these agents, which is otherwise attributed to their quinidine-like effect. However, the presence of an abundant amount of ubiquinone can overcome the difficulty posed by inhibition of those enzymes *(189)*. Riboflavin supplementation may be problematic because it may increase the autoinduction of amitriptyline.

7. MINERALS

The following discussion addresses the effect of medication on the status of electrolytes and trace elements (Tables 5 and 6). Keep in mind that disorders of sodium concentration must be evaluated in conjunction with volume status. For example, although a number of drugs can cause sodium retention (e.g., nonsteroidal anti-inflammatory drugs, corticosteroids, estrogens), there may be no influence on serum sodium concentrations because of simultaneous increases in water retention; however, edema might become apparent if retention is significant. Alterations of mineral status may be more clinically significant in the presence of renal dysfunction.

7.1. Absorption

Osteomalacia may result from poor mineralization of the bone during deficits of calcium and phosphorus as well as vitamin D. Several drugs, including cholestyramine, phenytoin, phenobarbital, isoniazid, aluminum-containing antacids, and rifampicin, may contribute to this adverse outcome by reducing absorption or altering metabolism and distribution *(190)*.

Aluminum-containing antacids can bind phosphate and fluoride within the gut, thereby reducing their absorption *(191)*. Formation of aluminum phosphates within the GI lumen accounts for the reduced phosphate absorption in the presence of aluminum-containing medication (e.g. antacids, sucralfate). In the past, this was used as a therapeutic approach to hyperphosphatemia in patients with chronic kidney disease. Long-term use of these binders can exacerbate osteomalacia that results in part from aluminum accumulation as well as the loss of phosphate and calcium.

Through their reduction of vitamin D, the anticonvulsants can decrease active intestinal calcium absorption, thereby placing these patients at risk for osteomalacia in the absence of vitamin D and calcium supplementation *(192)*. Reduction in serum calcium, along with increased alkaline phosphatase, has been noted to occur following the first month of phenytoin therapy *(193)*. Hypocalcemia may be more severe with phenobarbital treatment than with phenytoin or carbamazepine *(171)*. Valproic acid monotherapy is not associated with hypocalcemia.

Corticosteroids are also associated with loss of bone mass. This partly results from reduced vitamin D-dependent calcium absorption *(194)*.

Table 5
Drugs That Alter Macromineral Status

Sodium

Hyponatremia

- Acetazolamide
- Bromocriptine
- Carbamazepine
- Cyclophosphamide
- Chlorpropamide
- Clofibrate
- Fluoxetine
- Furosemide
- Heparin
- Hydrochlorothiazide
- Metolazone
- Morphine
- Oxytocin
- Pentamidine
- Sertraline
- Spironolactone

Hypernatremia

- Bumetanide
- Cidofavir
- Colchicine
- Demeclocycline
- Ethacrynic acid
- Foscarnet
- Furosemide
- Hypertonic Na salts
- Lithium
- Methoxyflurane
- Phenytoin
- Torsemide
- Vinblastine

Potassium

Hypokalemia

- Acetazolamide
- Activated charcoal
- Albuterol
- Amiloride
- Ammonium chloride
- Amphotericin B
- Ampicillin
- Aspirin
- Betamethasone
- Bisacodyl
- Bumetanide
- Carbenicillin
- Carboplatin
- Carmustine
- Chlorothiazide
- Chlorpropamide
- Chlorthalidone
- Cisplatin
- Colchicine
- Corticosteroids
- Corticotropin
- Cortisone
- Cyanocobalamin
- Cytarabine
- Desirudin
- Dexamethasone
- Dextrose
- Didanosine
- Digoxin immune fab
- Dobutamine
- Doxorubicin
- Ephedrine
- Epinephrine
- Ethacrynic acid
- Fluconazole
- Fluoxetine
- Foscarnet
- Furosemide
- Ganciclovir
- Gentamicin
- Hydrochlorothiazide
- Hydrocortisone
- Indapamide
- Insulin
- Isoflurane
- Isoproterenol
- Isosorbide mononitrate
- Itraconazole
- Levalbuterol
- Levodopa/Carbidopa
- Lithium
- Methylprednisolone
- Metolazone
- Mezlocillin
- Nafcillin
- Nifedipine
- Nylidrin
- Ondansetron
- Oxacillin
- Pamidronate
- Penicillin G
- Phosphates
- Piperacillin
- Polymyxin B
- Prednisolone
- Prednisone
- Pseudoephedrine
- Rifampin
- Risperidone
- Ritodrine
- Saline laxatives
- Salmeterol
- Sargramostim
- Sirolimus
- Sodium bicarbonate
- Sodium lactate
- Sodium polystyrene sulfonate
- Sorbitol
- Sotalol
- Tacrolimus
- Terbutaline
- Testosterone
- Theophylline
- Thiazides
- Ticarcillin
- Tobramycin
- Torsemide
- Triamcinolone
- Vincristine

(Continued)

Table 5 *(Continued)*

Hyperkalemia		
Amiloride	Glucagon	Propranolol
Atenolol	Heparin	Quinapril
Benazepril	Ibuprofen	Ramipril
Captopril	Indomethacin	Spironolactone
Cotrimoxazole	Metoprolol	Succinylcholine
Cyclosporine	Naproxen	Tacrolimus
Digoxin	Pentamidine	Triamterene
Enalapril	Potassium salts	
Magnesium		
Hypomagnesemia		
Albuterol	Docusate	Penicillamine
Amphotericin B	Estrogen	Pentamidine
Bumetanide	Ethacrynic acid	Phosphates
Carboplatin	Ethanol	Sargramostim
Chlorothiazide	Foscarnet	Sulfonamides
Cholestyramine	Furosemide	Tacrolimus
Cisplatin	Gentamicin	Tetracylines
Corticosteroids	Hydrochlorothiazide	Tobramycin
Cyclosporine	Insulin	Torsemide
Dextrose	Laxatives	Zoledronic acid
Didanosine	Oral contraceptives	
Digoxin	Pamidronate	
Hypermagnesemia		
Lithium		
Magnesium salts		
Phosphorus		
Hypophosphatemia		
Acetazolamide	Cisplatin	Insulin
Alendronate	Demeclocycline	Magnesium
Al-Mg antacids	Dextrose	Osmotic diuretics
Arginine	Digoxin	Pamidronate
Calcitonin	Erythropoietin	Sevelamer
Calcium salts	Ethanol	Sirolimus
Carmustine	Felbamate	Sucralfate
Cefotetan	Foscarnet	Tacrolimus
Cholestyramine	Glucagon	Zoledronic acid
Hyperphosphatemia		
Antileukemia regimens		
Phosphate salts		
Calcium		
Hypocalcemia		
Alendronate	Edetate disodium	Pamidronate
Amphotericin B	Estrogens	Pentobarbital
Antacids	Ethacrynic acid	Pentamidine
Bleomycin	Etidronate	Phenobarbital
Bumetanide	Famotidine	Phenytoin
Calcitonin	Fluocortolone	Phosphates

(Continued)

Table 5 *(Continued)*

Carboplatin	Fluoride	Polymyxin B
Cholestyramine	Fluorouracil	Propylthiouracil
Cisplatin	Foscarnet	Ranitidine
Cimetidine	Furosemide	Rituximab
Citrate Salts	Gentamicin	Saline laxatives
Codeine	Hydrochlorothiazide	Sargramostim
Corticosteroids	Interferon	Sodium polystyrene
Corticotropin	Isoniazid	Sulfonamides
Cyclosporine	Ketoconazole	Terbutaline
Cytarabine	Lansoprazole	Tetracyclines
Daunorubicin	Leucovorin	Tobramycin
Didanosine	Magnesium	Torsemide
Digoxin	Mineral oil	Triamterene
Diethylstilbestrol	Mithramycin	Zoledronic acid
Doxorubicin	Nizatadine	
Hypercalcemia		
Al-Mg antacids	Hydrochlorothiazide	Theophylline
Calcium salts	Lithium	Vitamin A
Ganciclovir	Tamoxifen	Vitamin D

The H_2 receptor antagonist cimetidine has the potential to lower serum calcium levels after several weeks of therapy without affecting parathyroid hormone (PTH) levels (probably by altering hepatic vitamin D metabolism inhibiting 25-hydroxylase activity) *(179,180,195–197)*. As a result of decreasing gastric acid, this class of agents may reduce the absorption of dietary iron and place patients at risk for iron deficiency *(77)*. However, this finding was not confirmed in patients with Zollinger-Ellison Syndrome who were chronically treated with H_2 antagonists or proton pump inhibitors *(198)*. Managing this risk through iron supplementation may actually lead to decreased drug and iron absorption (at least in the case of cimetidine), because a drug–nutrient complex is formed; however, any reduction in drug absorption may not be clinically relevant *(199,200)*. Absorption of zinc is also reduced in individuals who are treated with cimetidine *(201)*.

Cholestyramine may negatively influence the status of magnesium, calcium, iron, and zinc *(202)*. The absorption of multivalent cations (e.g., calcium, iron, magnesium) may be reduced by chelating with the tetracycline antibiotics *(203)*. Malabsorption of sodium and potassium can occur as a result of the GI effects of colchicine and laxatives.

7.2. Distribution

Intracellular concentrations of potassium and magnesium are reduced in patients who are treated with diuretics *(204)*. This may occur without a reduction in the serum concentration and despite potassium supplementation.

Magnesium depletion can occur in tissues of patients who are receiving thiazide diuretics without altering serum magnesium concentrations *(205)*. Magnesium losses are especially important in light of the poor dietary intake of magnesium among adults *(206)*. However, thiazide diuretics do cause calcium retention, which may even improve bone mineral content in chronic users, translating to lower risk of hip fractures *(207,207a)*.

Table 6
Drugs That Alter Trace Mineral Status

Chromium	
Corticosteroids	
Copper	
Antacids	Penicillamine
Antivirals	Valproic acid
Corticosteroids	Zidovudine
Ethambutol	Zinc Salts
Oral contraceptives	
Iron	
Aspirin	Ethanol
Calcium	Indomethacin
Cholestyramine	Neomycin
Deferoxamine	Sulfonamides
Erythropoietins	Tetracyclines
Selenium	
Corticosteroids	
Valproic Acid	
Zinc	
Antivirals	Ethambutol
Captopril	Folic acid
Cholestyramine	Hydrochlorothiazide
Cimetidine	Oral contraceptives
Corticosteroids	Penicillamine
Deferiprone	K-sparing diuretics
Edetate Ca disodium	Thiazides
Enalapril	Valproic acid
Estrogen	Zidovudine

Thiazide diuretics, selective serotonin reuptake inhibitors, and others are more likely to cause hyponatremia in the elderly as a result of impaired water excretion.

By inhibiting bone resorption, bisphosphonates (e.g., alendronate) may cause or exacerbate uncorrected hypocalcemia in the absence of adequate calcium and vitamin D intake. Patients with tumor-related hypercalcemia can be treated with pamidronate to lower serum calcium levels. However, hypocalcemia may occur in about one-fourth of patients who are treated with pamidronate, although they are usually asymptomatic *(208)*.

Estrogen use can cause a decrease in serum magnesium (although not as much as is seen in pregnancy), which has been suggested to result from intracellular shifting *(209,210)*. Low dietary magnesium intake in women using oral contraceptives or estrogen replacement, particularly in the presence of calcium supplementation, may increase the risk of adverse drug effects, including thrombosis *(211)*. The effect of oral contraceptives on copper and zinc are widely described, but the mechanism for this effect remains unclear *(126,144)*. Both whole blood and plasma levels of copper increase in patients using oral contraceptives, whereas the data regarding decrease of zinc concentrations is much less consistent *(212–214)*.

Corticosteroids can cause a dose-dependent, transient decrease in serum zinc concentrations following an initial rise *(215)*. No decrease was identified after 48 h following

an intravenous dose. A slower decline in serum copper levels that is attributed to corticosteroids may last as long as 96 h after a dose *(215)*. Serum selenium levels have been noted to increase during corticosteroid treatment *(216)*. The clinical significance of the redistribution of these minerals with corticosteroid administration is not clear but may play a role in immunosuppression.

Patients treated with zidovudine were noted to have reduced serum copper and zinc levels compared to similar patients who were not receiving this antiretroviral medication *(217)*. Although it has only been investigated in an animal model, the drug ethambutol may decrease distribution of copper to the liver and heart and distribution of zinc to the liver and kidneys *(218)*.

7.3. Excretion

7.3.1. Electrolytes

Electrolyte loss may occur through the GI tract or the kidneys. Sodium, potassium, and water depletion following chronic use of laxatives has been well-recognized for years as the so-called "laxative abuse syndrome" but still continues to occur in patients *(219,220)*. Elevated levels of renin and aldosterone respond well to potassium, sodium, and fluid repletion *(221)*. Prophylactic potassium supplementation may be beneficial in patients requiring bowel regimens who may have a history of cardiovascular disease *(222)*. Of course, appropriate use of laxatives is the best management approach in this case. Besides increasing the risk for hyperphosphatemia, phosphate enemas can cause hypocalcemia and hypomagnesemia as well as hypokalemia *(223–225)*. The phosphate load of the concentrated product is excessive—particularly for infants, the elderly, and others with impaired renal function.

A number of drugs can increase renal losses of electrolytes (e.g., diuretics, antimicrobials, chemotherapy). The pharmacological activity of most diuretics involves the renal excretion of sodium. Loop diuretics increase renal wasting of potassium and magnesium *(226,227)*; the latter likely occurs through an inhibition of passive magnesium absorption *(228)*. Magnesium wasting occurs with a number of other drugs *(229)*. Magnesium deficits may exist in the face of serum magnesium concentrations within the normal range. In fact, unrecognized drug-induced hypomagnesemia may be responsible for refractory episodes of hypokalemia and hypocalcemia. Loop diuretics also increase loss of calcium in the urine as PTH levels increase *(230)*. Loss of all these electrolytes through diuresis increases the risk of arrhythmia and sudden death in patients treated with these agents *(231,232)*.

The penicillins (e.g., carbenicillin, ampicillin, nafcillin, ticarcillin, oxacillin) are reported to cause hypokalemia, probably because of increased potassium secretion resulting from the solute load (nonreabsorbable anion) presented to the distal renal tubules *(233–237)*. Hypocalcemia, hypokalemia, and hypomagnesemia all have been noted in patients treated with aminoglycoside antibiotics *(238)*. Treatment with the antifungal agent amphotericin is known to result in renal depletion of potassium and magnesium. Hypokalemia, in turn, may potentiate the tubular toxicity of amphotericin *(239)*. This may be less likely in patients treated with liposomal formulations of this drug *(240)*. Foscarnet can result in hypokalemia, hypomagnesemia, hypocalcemia, and hypophosphatemia *(229,241,242)*. The effect of foscarnet on calcium and phosphorus may be minimized when the drug is administered as a liposome-encapsulated formulation *(243)*. Foscarnet binding to ionized calcium may explain some of the abnormalities *(244)*.

Corticosteroids—administered orally or parenterally—increase the risk of hypokalemia in patients receiving diuretics *(245)*. Urinary potassium excretion increases with dose and duration of corticosteroid therapy *(246)*. Mineralocorticoids stimulate renal tubular potassium secretion directly, whereas glucocorticoids do so indirectly through the sodium load presented to the distal tubule *(247)*. Corticosteroids may also increase renal magnesium losses, resulting in hypomagnesemia *(248)*. Corticosteroids can decrease intestinal calcium absorption, increase renal calcium excretion, and decrease osteoblast activity, all of which contributes to osteoporosis. This drug-induced osteoporosis may partly depend on the dose and duration of corticosteroid use as well as bone mineral density prior to initiation of the drug regimen. Supplementation with vitamin D and calcium can prevent some of the increases in PTH and loss of bone mineral density that occur with corticosteroid therapy *(249,250)*. The altered calcium absorption may vary among glucocorticoids through a mechanism that apparently does not involve 1,25-dihydroxy-cholecalciferol *(251)*. The proton pump inhibitor lansoprazole has been reported to cause severe, symptomatic hypocalcemia through an unknown mechanism *(252)*. Other immunosuppressants besides corticosteroids also can cause electrolyte disturbances. Cyclosporine and tacrolimus can cause increased renal magnesium losses and possible potassium losses as well *(253,254)*.

A number of medications, including the selective serotonin reuptake inhibitors, can cause hyponatremia (particularly in the elderly) as a result of increased secretion of antidiuretic hormone *(255)*. Other medications, including lithium therapy, can cause hypernatremia as a consequence of decreased renal responsiveness to antidiuretic hormone (i.e., nephrogenic diabetes insipidus) *(256–258)*. In either case, the alteration in serum sodium concentration is related to a disorder of water regulation, which either dilutes (hyponatremia) or concentrates (hypernatremia) the sodium content of the serum. Therefore, management of these disorders includes water restriction (hyponatremia) or water replacement (hypernatremia). If the hypernatremia associated with lithium is managed with sodium restriction rather than water replacement, the risk of lithium toxicity is actually increased as the drug is reabsorbed renally in place of sodium.

7.3.2. Trace Minerals

Loss of blood at the GI tract is higher as a result of aspirin or other nonsteroidal anti-inflammatory drug therapy and may increase the risk of developing iron deficiency *(259,260)*. Aspirin use has been associated with lower serum ferritin concentrations in some patients, although confounding factors could have played a role *(261)*.

Captopril and, to a lesser extent, enalapril increase urinary zinc excretion after several months of treatment in patients with hypertension *(262,263)*. This is not reflected by serum values of zinc, which remain unchanged *(264)*. No documentation exists regarding whether this will lead to clinically apparent deficits, but it may play a role in the altered taste experienced by patients receiving this class of medication. Diuretics (including the thiazides and chlorthalidone) increase urinary zinc losses *(265,266)*. Again, serum levels remain normal in most patients, although this is expected as a result of redistribution from the intracellular space.

Penicillamine is used to lower serum copper concentrations in patients with Wilson's Disease by increasing renal elimination of a drug–mineral complex. Although penicillamine is useful in Wilson's Disease, this nutrient loss may explain the drug's teratogenic effect *(267)*. Penicillamine also has the potential to lower levels of other nutrients. Loss of zinc may be increased, but iron, calcium, and magnesium losses are

not readily apparent *(268)*. Circulating zinc levels improve within a few months of continued therapy; it is not clear whether this is a recovery of zinc status or merely a redistribution from other compartments *(269)*. Supplementation of magnesium, zinc, and pyridoxine has been suggested for patients receiving penicillamine *(270)*. Zidovudine and valproic acid may also reduce serum copper concentrations *(271)*.

Additionally, corticosteroids can increase chromium losses and, therefore, may contribute to the hyperglycemia associated with these agents *(272)*. Corticosteroids may also increase urinary loss of selenium *(273)*. Understanding the clinical relevance of drug-induced losses of the trace minerals requires additional study.

8. SUMMARY AND RECOMMENDATIONS

A nutritionally focused patient history, physical examination, and appropriate laboratory marker evaluations are important to correctly identify nutrient deficits or excesses in patients using medication. No single clinician specifically looks for nutritional aspects of drug use on a regular basis. Any change in nutritional status identified by a clinician should be examined for a drug-induced etiology.

Everyone requiring medication should have a thorough nutritional assessment performed at baseline and periodically during chronic treatment. Any abnormalities in nutritional status or status of a specific nutrient identified by this diligence should be documented and published to educate others. For those drugs known to cause altered nutritional status, specific interventions should occur at the time the medication is started to prevent adverse effects. When a drug-induced change poses a significant concern for a patient, a therapeutically equivalent or alternative agent may be selected.

Current evidence does not support indiscriminant use of nutrient supplements to manage losses that may result from drug use. Indeed, the dose of a nutrient that is needed to offset the adverse effects of many medications is not known. Additionally, in some instances, pharmacological nutrient dosing may increase drug clearance with clinical consequences, as in the case of folic acid and phenytoin *(274)*.

The clinical relevance of the drug-induced changes to the status of an individual nutrient needs to be determined on an individual basis. This relevance may be viewed as a finding of little value or may help to explain debilitating adverse effects associated with chronic use of a medication. Aside from the few widely recognized interactions, most of these interpretations usually are not made.

Ideally, all new drugs should be evaluated for effects on nutritional status. Clinicians should be sensitized to identify and report effects of drugs that are otherwise unrecognized and should develop guidelines to address altered nutritional status that take into account the most appropriate test(s) of status. Prospective studies regarding methods to correct nutritional status should evaluate not only parameters of nutrient status but influence on clinical manifestations. In this way, patients required to use medication can be optimally managed regarding maintenance of adequate nutritional status.

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35 Health Claims for Foods and Dietary Supplements

Current and Emerging Policies

Annette Dickinson

KEY POINTS

- There is scientific consensus that improving dietary habits helps to promote health and reduce the risk of chronic diseases.
- Scientifically substantiated health claims in food labeling can help consumers identify healthy foods and food habits that may protect health and prevent disease.
- The Nutrition Labeling and Education Act of 1990 authorized the US Food and Drug Administration (FDA) to approve health claims for foods and dietary supplements when the claims were supported by "significant scientific agreement," and a number of such claims have been approved.
- Courts have ruled that the FDA must also consider permitting "qualified" health claims when such claims are truthful and not misleading, and the agency has permitted a number of qualified health claims.
- The Dietary Supplement Health and Education Act of 1994 authorized marketers of dietary supplements to use label statements describing the effect of a product on the structure and function of the body (under certain conditions), and these "structure/function" statements are widely used.
- The interest in health claims is global, but different countries and international policy-making bodies have adopted markedly different regulatory structures for handling such claims.

1. INTRODUCTION

Although there is widespread scientific acceptance of the importance of diet in reducing the risk of chronic disease, there is continuing controversy about the extent to which statements about diet and health may be appropriately included in food product labeling and advertising. The United States has undertaken major initiatives in exploring mechanisms for harnessing the power of commercial product labeling to convey or reinforce government-sanctioned health messages about specific types of foods or food components, and other countries and international organizations also are exploring

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Table 1
Abbreviations Used in This Chapter

Ca	Calcium
CDC	Centers for Disease Control and Prevention
CHD	Coronary heart disease
DRI	Dietary Reference Intake
DSHEA	Dietary Supplement Health and Education Act (1994)
FDA	Food and Drug Administration
FDAMA	Food and Drug Administration Modernization Act (1997)
FOSHU	Food for Specified Health Uses (Japan)
FTC	Federal Trade Commission
NCI	National Cancer Institute
NLEA	Nutrition Labeling and Education Act (1990)
NRC	National Research Council
NTDs	Neural tube birth defects
RDA	Recommended Daily (Dietary) Allowances
RDI	Reference Daily Intake
ω-3	Polyunsaturated fatty acids

alternatives for permitting health-related statements in the labeling of conventional foods and dietary supplements. Over the past decade, public policy regarding health claims for food has evolved based on complex legal and scientific considerations. A list of abbreviations used in this chapter appears in Table 1.

2. PUBLIC HEALTH RECOMMENDATIONS IN THE UNITED STATES

Scientific and public acceptance of the existence of a strong relationship between diet and health has been growing for more than two decades in the United States and worldwide. An early and influential compilation of the data appeared in 1977, when the Senate Select Committee on Nutrition and Human Needs published its report on Dietary Goals for the United States *(1)*. The report included recommendations for dietary patterns that might decrease the risk of several "killer diseases," such as cancer and heart disease. Although there was opposition to the dietary goals from some quarters, the concept of public recommendations for dietary changes that lead disease prevention soon became firmly established.

Following publication of the report by Senate Select Committee, Congress directed the Department of Health and Human Services and the United States Department of Agriculture to prepare a set of dietary guidelines for reducing the risk of chronic disease. The first edition of Dietary Guidelines for Americans appeared in 1980 and, by Congressional directive, has been re-evaluated and republished every 5 yr thereafter *(2)*. The sixth set of these guidelines is currently being developed by an expert committee and will be finalized before the end of 2005.

In 1979, the Surgeon General's Report on Health Promotion and Disease Prevention, Healthy People, set forth a broad agenda for preventive actions that could be taken to reduce health risks, including some general nutritional recommendations similar to those suggested by the Senate Select Committee *(3)*. This report ultimately led to the development of a series of wide-ranging US action plans that call on federal, state, and local governments as well as other public and private organizations to take cooperative

action to measurably reduce recognized risk factors and to increase health-promoting behaviors. Objectives have been defined for the third decade of the program—Healthy People 2010 *(4)*.

In 1982, the National Research Council (NRC) published a report called Diet, Nutrition, and Cancer, which outlined specific dietary recommendations for cancer reduction *(5)*. The report called for increased consumption of fruits and vegetables that are rich in antioxidant vitamins, cruciferous vegetables, and grains and other foods that are rich in fiber. It emphasized that these recommendations were aimed at increasing intakes of certain food groups or types of foods—not specific nutrients.

In 1988 and 1989 the Surgeon General's report on on *Nutrition and Health* and the NRC report on *Diet and Health*, respectively, reviewed the data and made broad recommendations regarding dietary habits that were likely to help reduce the risk of cancer, heart disease, hypertension, diabetes, obesity, osteoporosis, and other chronic diseases *(6,7)*.

In 1994, the Food and Nutrition Board of the National Institute of Medicine published a thoughtful document regarding the philosophical and scientific basis for the Recommended Daily (Dietary) Allowances (RDAs) and indicated that, for the first time, a document existed to evaluate the potential for chronic disease prevention as well as classic measures of nutritional status in establishing new recommendations *(8)*. That effort is now complete and has already resulted in reports that provide recommendations for six categories of nutrients *(9–14)*. These reports establish Dietary Reference Intakes for specific nutrients, potentially including estimated average requirements, adequate intakes, RDAs, and upper levels of tolerable intake. Reports cover calcium and related nutrients, B-vitamins, antioxidant nutrients, trace nutrients, and macronutrients, and electrolytes. The reports include extensive discussions of the potential for disease reduction that is related to increased intakes of many nutrients; however, in most cases, the recommended intakes are based on traditional measures of nutritional status rather than on disease prevention. However, substantially increased intakes of calcium and vitamin D are recommended, especially for older adults, to help enhance bone density and reduce the risk of osteoporosis (*see* Chapter 18). The report on B-vitamins urges women of childbearing age to get 0.4 mg (400 μg) of *synthetic* folic acid per day in addition to their usual dietary folate intake to reduce the risk of having a baby with neural tube defects (NTDs) (*see* Chapter 24). The evidence on antioxidants and disease prevention is insufficient to form the basis for new and higher intake recommendations, but more research is encouraged (*see* relevant chapters in the sections on cancer and cardiovascular disease.)

As these various examples indicate, US public health policy regarding diet and health has been moving steadily in the direction of putting increased emphasis on the potential for reducing the risk of chronic disease through dietary changes.

3. THE US FOOD MARKETPLACE AND THE LAW

Although the scientific community was developing new dietary recommendations intended to reduce disease risk, food marketing companies both in the United States and globally were constrained in their ability to make use of this information in labeling and advertising. As discussed later, disease-related statements in food labeling were officially viewed by the US Food and Drug Administration (FDA) as inappropriate or illegal, and the food industry was accordingly restricted in promoting health benefits.

In 1984, the National Cancer Institute (NCI) was pursuing various educational programs to increase public awareness of dietary patterns that might reduce cancer risk. As part of this initiative, NCI entered into a joint national campaign with the Kellogg Company to promote diets that were high in fiber. Informational messages appeared prominently in the labeling of certain of Kellogg's high-fiber cereals.

The US Food, Drug, and Cosmetic Act defines drugs, in part, as products "intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals." Under this law, claims relating to disease prevention were viewed historically as drug claims and were not permitted in food labeling. However, in 1984, faced with Kellogg's cereals that bore NCI-supported claims about fiber and cancer, the FDA entered into a public dialogue about the desirability of permitting "health claims" for foods under certain conditions.

Several forces were at work during this period. The food industry was increasingly anxious to use the strong science base to promote foods with desirable characteristics. Consumer advocates were concerned that claims were getting out of control and urged Congress to permit only those claims that were specifically authorized by the FDA under strict guidelines. In 1989, the Federal Trade Commission (FTC) released a report concluding that fiber claims in the marketplace had both increased consumer awareness of the relationship between fiber and cancer and increased the number of high-fiber products that were available to the public *(14)*. Both of these outcomes were viewed as positive developments by the authors of the FTC report. The FDA began the process of developing guidelines (or regulations) for health claims, but some legal questions persisted about whether the FDA had the authority to permit health claims for foods without a change in the statute.

In 1990, Congress passed the Nutrition Labeling and Education Act (NLEA), which gave the FDA clear authority to permit health claims in food labeling under certain conditions. (NLEA also put mandatory nutrition labeling in place and defined nutrient content claims, but neither of these aspects of NLEA are discussed in this chapter.) A health claim is defined as a label statement characterizing the relationship of a nutrient or food component to a disease or health-related condition. Under the new law, foods with approved health claims are not considered "drugs" solely because their labeling contains a health claim, although by definition, the health claim must mention a disease or health-related condition.

To approve an NLEA health claim, the FDA must determine, "based on the totality of publicly available scientific evidence (including evidence from well-designed studies conducted in a manner which is consistent with generally recognized scientific procedures and principles), that there is significant scientific agreement, among experts qualified by scientific training and experience to evaluate such claims, that the claim is supported by such evidence." Also, the FDA must issue a regulation describing required components of the claim to ensure that the claim accurately conveys the nutrient–disease relationship. The NLEA specified that the claim should enable consumers "to comprehend the information provided in the claim and to understand the relative significance of such information in the context of a total daily diet."

The NLEA provided the FDA with a list of 10 health claims that the agency was required to evaluate initially, including 4 designated as claims for dietary supplements. The six core claims listed in the NLEA related to fat and cancer, fat and heart disease, sodium and hypertension, fiber and cancer, fiber and heart disease, and calcium and osteoporosis. The four claims listed as dietary supplement claims were for antioxidant

vitamins and cancer, folic acid and NTDs, ω-3 fatty acids and heart disease, and zinc and immune function in the elderly.

4. US HEALTH CLAIMS REGULATIONS

In January 1993, the FDA finalized regulations establishing "general requirements" for health claims *(15)*. For a food to be eligible for a health claim, it must not contain disqualifying amounts of any "risk nutrients," including total fat, saturated fat, cholesterol, or sodium. Also, a serving of the food must naturally contain at least 10% of the RDA of vitamins A and C, iron, calcium, protein, or fiber. This provision, sometimes referred to as the "jelly bean rule," is intended to ensure that foods that bear health claims are products with some inherent nutritional value. If a health claim suggests that people should consume more of any substance (such as calcium or folic acid or fiber), the FDA must make a determination that the substance is safe and lawful. Additionally, the substance must contribute taste, aroma, nutritive value, or a designated technical effect in foods and must retain those qualities when consumed at levels high enough to justify the health claim. In the regulation authorizing a health claim, the FDA provides "model claims" that may be used in product labeling. Marketers are not limited to the language of the "model claim," but the language they use must convey certain information specified in the regulation.

In 1993, the FDA issued separate regulations approving three health claims for foods that were low in certain components that were related to disease risk *(16–18)*. These included claims that foods low in lipids may help reduce the risk of cancer, that foods low in saturated fat and cholesterol may help reduce the risk of coronary heart disease (CHD), and that foods low in sodium may help reduce the risk of hypertension.

Meanwhile, the FDA approved three health claims for foods that were naturally good sources of dietary fiber, soluble fiber, or two of the antioxidant vitamins *(19–21)*. In all three cases, the FDA argued that the available evidence indicated that diets that were high in these nutrients were protective against cancer and/or heart disease but did not permit a determination that these nutrients were the cause of the protective effect (i.e., the FDA concluded that fiber and antioxidant vitamins may be only markers for protective foods or disease-reducing food habits). Under these regulations, health claims about reduction of cancer risk may be made for fruits, vegetables, and grains that are low in fat and are naturally good sources of dietary fiber, vitamin C, or vitamin A from β-carotene. Health claims also may be made about the reduction of CHD risk by fruits, vegetables, and grains that are low in fat, saturated fat, and cholesterol and that contain at least 0.6 g of soluble fiber per serving.

The FDA declined to approve any health claims for specific fiber sources in 1993, but the agency indicated that it would be willing to review health claim petitions based on sufficient evidence showing that specific fibers lower cholesterol. In 1997 and 1998, the FDA approved petitions for health claims for oat fiber and psyllium fiber *(22,23)*. The oat claim is permitted for foods (including dietary supplements) that contain a sufficient amount of oat bran, rolled oats, or whole oat flour to provide at least 0.75 g of β-glucan-soluble fiber per serving. The psyllium claim is permitted for foods (including dietary supplements) that contain at least 1.7 g of psyllium fiber per serving. In 1999, the FDA approved a health claim for soy protein; this claim was also approved on the grounds that soy protein lowers cholesterol, and therefore, may reduce the risk of heart

disease *(24)*. The claim is permitted for foods that are low in saturated fat and cholesterol and that contain at least 6.25 g of soy protein per serving. A health claim has also been permitted for foods containing stanol or sterol esters, which lower cholesterol and thus may reduce the risk of heart disease *(25)*.

In 1993, in addition to the three claims for foods low in "risk nutrients" and the three claims for food groups naturally high in some beneficial nutrients, the FDA also approved one claim that could appear on supplements and fortified foods as well as unfortified conventional foods. This was the calcium health claim, which may be used in the labeling of a food or dietary supplement that meets the general requirements for health claims and contains at least 200 mg of calcium per serving *(26)*. The claim indicates that diets that are high in calcium may reduce the risk of osteoporosis.

In all of the 1993 regulations that approved health claims, the FDA relied heavily on the Surgeon General's Nutrition and Health report, the NRC Diet and Health report, and the *Dietary Guidelines for Americans* as evidence of "significant scientific agreement" regarding specific diet and health relationships. Health claims for diet–disease relationships that were not favorably considered in these reports were disapproved, including claims for ω-3 fatty acids and heart disease and for zinc and immune function in the elderly *(27,28)*. Disapproval of a health claim was interpreted to mean that no statements about the diet–disease relationship could be made in food or dietary supplement labeling—not even a statement that accurately described the state of the emerging evidence. This interpretation was challenged in subsequent litigation, as will be discussed later.

In 1996, the FDA approved a health claim petition that foods sweetened with sugar alcohols were related to a reduced risk of dental caries *(29)*. The rule permits the use of xylitol, sorbitol, mannitol, maltitol, isomalt, lactitol, hydrogenated starch hydrolysates, hydrogenated glucose syrups, or a combination thereof. In 1997, erythritol was added to the list of sugar alcohols that are eligible for this health claim *(30)*.

The health claim that was the subject of the greatest controversy involved the relationship between the B-vitamin folic acid and a reduced risk of giving birth to a baby with a NTD. In 1997, the delay in approving the folic acid health claim eventually led to the development of an alternative procedure for permitting health claims. In 1992, the US Public Health Service issued a recommendation that urged women of childbearing age to obtain 0.4 mg of folic acid daily before conception and early in pregnancy to reduce the risk of having a baby with a NTD such as spina bifida or anencephaly *(31)*. The development of this recommendation was spearheaded by scientists at the Centers for Disease Control and Prevention (CDC). Although the FDA, as part of the Public Health Service, participated in the development of this recommendation, in 1993, the agency declined to authorize a health claim for folic acid *(32)*, because it was considering requiring mandatory enrichment of grain products with folic acid and was concerned about potential safety issues if some individuals obtained additional folic acid from other sources, including foods and dietary supplements that bore health claims.

The FDA did not officially approve a health claim for folic acid until 1996 *(33)*. The agency indicated that the safety issues had been resolved, because an enrichment plan had been developed that would maintain total folic acid intake within safe ranges. The health claim is permitted in the labeling of any foods or supplements that provide at least 10% of the Reference Daily Intake (RDI) of folic acid. This relatively low level was selected as the basis for the suggestion to permit the claim to appear on the largest

Table 2
NLEA Health Claims Approved by the US Food and Drug Administration as of September 2004

Food	*Claim*
Health claims for foods low in certain components	
Foods low in total fat and saturated fat	Reduced cancer risk
Foods low in saturated fat and cholesterol	Reduced risk of CHD
Foods low in sodium	Reduced risk of hypertension
Health claims for foods naturally containing certain components	
Foods that are good sources of fiber	Reduced risk of cancer
Foods that are good sources of soluble fiber	Reduced risk of CHD
Foods that are good sources of vitamin C or β-carotene	Reduced risk of cancer
Health claims for specific food components, whether added or naturally occurring	
Foods high in calcium	Reduced risk of osteoporosis
Foods with folate or folic acid	Reduced risk of having a baby with NTDs
Foods sweetened with sugar alcohols	Reduced risk of dental caries
Foods with whole oats or oat bran containing β-glucan	Reduced risk of heart disease
Foods with psyllium fiber	Reduced risk of heart disease
Foods with soy protein	Reduced risk of heart disease
Foods with stanol and sterol esters	Reduced risk of heart disease

Abbreviations: CHD, coronary heart disease; NTD, neural tube defect.

possible number of fruits and vegetables. Although this same low trigger level applies in defining the eligibility of a dietary supplement for this health claim most dietary supplements that contain folic acid provide the full RDI of 0.4 mg (400 μg). The regulation also requires that dietary supplements bearing the folic acid health claim must meet disintegration and dissolution standards established by the US Pharmacopeia or must be shown to be bioavailable.

Simultaneously with the finalization of the folic acid health claim, the FDA also finalized a series of rules amending the Standards of Identity for enriched grain products to require mandatory addition of folic acid, beginning in 1998 *(34)*. Products that were affected included enriched flour, corn meal, breads, rice, and pasta. The agency also amended the food additive rule on folic acid to define other permitted uses of folic acid in foods *(35)*. The revised rule permitted breakfast cereals to contain up to 0.4 mg of folic acid per serving and also permitted addition of folic acid to infant formula, medical foods, and foods for special dietary use; other addition of folic acid to foods is not allowed.

Table 2 is a summary list of the NLEA health claims that had been approved by the FDA as of September 2004.

5. FOOD AND DRUG ADMINISTRATION MODERNIZATION ACT OF 1997 HEALTH CLAIMS

The FDA Modernization Act of 1997 (FDAMA) was a major legislative initiative that had broad effects on the regulation of drugs and devices and on FDA approaches to priority-setting and stakeholder involvement in the decision-making process. Among its many provisions, FDAMA also created an alternative route for permitting health claims

for foods. The FDA delay in approving the folic acid health claim was frequently and strongly criticized. Marketers argued that they should have been able to base a health claim on the 1992 Public Health Service recommendation, despite the absence of FDA approval of a formal health claim for folic acid. This argument found a sympathetic ear in Congress; therefore, FDAMA contains a provision that permits manufacturers to make health claims based on authoritative statements by certain scientific bodies, including the National Institutes of Health, the CDC, and the National Academy of Sciences. Before making a health claim based on an authoritative statement by an appropriate scientific body, a company must notify the FDA 120 d in advance. This provides the FDA with an opportunity to evaluate the claim and to deny it or require modifications if it is found not to be valid.

In 1998, the FDA denied an early petition for nine FDAMA health claims, largely because the statements cited were not authoritative statements of scientific bodies *(36)*. In 1999, the FDA allowed the use of the first FDAMA health claim—a health claim submitted by General Mills based on the *Diet and Health* report from 1989 by the National Academy of Sciences. The claim states that the consumption of whole grain foods is related to a reduced risk of heart disease and some cancers. In 2000, the FDA allowed a health claim submitted by Tropicana that suggested that foods that are good sources of potassium and are low in sodium may reduce the risk for high blood pressure and stroke; this claim was also based on the 1989 *Diet and Health* report.

6. COURT OF APPEALS DECISION

Some may be surprised to learn that government regulations (including FDA regulations) can raise constitutional issues. The first amendment to the United States Constitution guarantees freedom of speech, and the courts have held that this protection extends to not only to individual speech but to commercial speech as well (such as labeling and advertising). The government can prohibit false or misleading commercial speech but is constrained in its ability to restrict speech that is truthful and not misleading. In the case of health claims, the FDA does not allow the use of fully authorized NLEA health claims unless they are supported by "significant scientific agreement." Qualified health claims (health claims with disclaimers about the strength of the evidence) also were not considered permissible, even if they truthfully described the level of scientific support for a particular diet–disease relationship.

Several parties brought suit against the FDA for violating the first amendment by denying certain health claims. The first successful case was argued by attorney Jonathan Emord on behalf of a coalition of petitioners, including Durk Pearson and Sandy Shaw (authors who are also developers and marketers of some dietary supplement products), the American Preventive Medical Association (a health rights advocacy organization whose members are health care practitioners), and Citizens for Health (a health rights advocacy organization whose members are consumers). In this case, *Pearson v. Shalala*, in January 1999, a US Court of Appeals ruled that some aspects of the FDA current health claims approval process may have violated the first amendment as well as the Administrative Procedure Act *(37)*. The court concluded that the FDA needed to better define the term "significant scientific agreement" and at least consider the appropriateness of permitting "qualified" health claims. For example, the FDA rejected specific health

claims for antioxidants because the evidence suggested a beneficial effect of certain types of foods, but the component responsible for the effect was not definitive. The court suggested that rather than banning the claim for antioxidant vitamins outright, the FDA should have considered permitting a claim with a required disclaimer, such as: "The evidence is inconclusive because existing studies have been performed with foods containing antioxidant vitamins, and the effect of those foods on reducing the risk of cancer may result from other components in those foods."

In this ruling, the court cited legal precedents that overturned government regulations "that seek to keep people in the dark for what the government perceives to be their own good." The court suggested that more, rather than less, information may be the solution. At the same time, the court recognized that when the evidence against a claim outweighs the evidence in its favor, the FDA may choose to ban it outright (i.e., a disclaimer cannot salvage a claim that is basically misleading). Regulations denying substance-specific health claims for dietary fiber and cancer, antioxidant vitamins and cancer, and ω-3 fatty acids and CHD were remanded to the FDA with instructions for reconsideration. The agency was also instructed to reconsider some aspects of the folic acid health claim.

In December 1999 and January 2000, the FDA announced its plan for implementing the court's decision, issued a guidance document that better defined "significant scientific agreement," and re-opened the comment period on the four claims at issue *(38–40)*. Although the agency was reconsidering the evidence on those claims, attorney Jonathan Emord submitted additional health claims for consideration, including claims relating to vitamin E and heart disease, B-vitamins (folic acid and vitamins B_6 and B_{12}) and cardiovascular disease (CVD), and saw palmetto and benign prostatic hyperplasia.

In its decision to permit some qualified health claims, the FDA adopted the approach of "exercising enforcement discretion" by not challenging the claims, rather than approving them outright. Notification of the agency's decision is provided in the form of a letter to the petitioner who has requested the health claim, and the letter is posted on the FDA website. No regulation is adopted regarding the qualified health claim. In this manner, the FDA agreed to permit qualified health claims relating to ω-3 fatty acids and heart disease, B-vitamins and CVD, and some aspects of the claim relating to folic acid and NTDs. The FDA provided model language, which it considered to be appropriate for each claim. For example, in the ω-3 claim, the agency suggested the following model language: "Consumption of ω-3 fatty acids may reduce the risk of CHD. FDA evaluated the data and determined that, although there is scientific evidence supporting the claim, the evidence is not conclusive."

The agency declined to permit qualified health claims for antioxidants and cancer or for vitamin E and heart disease, because the weight of the scientific evidence was not supportive of a beneficial effect. The FDA also denied any health claim for saw palmetto and benign prostatic hyperplasia, because the claim related to a treatment effect in cases where hyperplasia already existed, rather than a preventive or risk reduction effect in healthy men.

Emord appealed the FDA denial of the antioxidant health claim, arguing that the agency had overinterpreted the degree of scientific support that was required for a qualified health claim. The agency reviewed more than 150 studies and found that the evidence against the claim outweighed the evidence in favor of it. The FDA concluded that the claim would be inherently misleading to a degree that could not be cured by a disclaimer. In December

2002, a United States District Court ruled against the FDA, noting that approximately one-third of the studies that the FDA examined were supportive of the antioxidant claim and that the "FDA's decision to ban the claim completely is not in accordance" with the Court of Appeals' findings in *Pearson v. Shalala (41)*. The court instructed the FDA to craft one or more "short, succinct, and accurate disclaimers" to be used in the context of a qualified health claim about antioxidants and cancer risk. The FDA subsequently agreed to permit a qualified health claim, such as: "Some scientific evidence suggests that consumption of antioxidant vitamins may reduce the risk of certain forms of cancer. However, FDA has determined that this evidence is limited and not conclusive" *(42)*.

Emord appealed the FDA denial of the saw palmetto health claim, suggesting that the NLEA defines a health claim as a statement describing the relationship between a nutrient and a disease or health-related condition and does not specify that the relationship must be preventive in nature. However, the FDA's denial of the claim was upheld by the Court of Appeal *(42a)*.

7. THE FOOD AND DRUG ADMINISTRATION'S INITIATIVE ON CONSUMER HEALTH INFORMATION FOR BETTER NUTRITION

The FDA approach to the evaluation and approval of health claims in the decade following the passage of the NLEA (1990) was highly conservative. Mark B. McClellan took office in November 2002 as the new Commissioner of the FDA. McClellan is a physician and an economist who quickly adopted a more proactive view of the FDA role in regulating the flow of health information to the consumer. In December 2002, he announced the formation of a Task Force on Consumer Health Information for Better Nutrition; in July 2003, he released a Task Force report establishing new groundrules for the review of health claims—especially qualified health claims *(42)*. The FDA emphasized that this new approach would provide consumers with "better, easily understood, up-to-date scientific information about how dietary choices can affect health." The agency stated that it would encourage marketers of conventional foods and dietary supplements to compete on the basis of health and nutrition benefits in addition to other product characteristics, such as taste and convenience. The FDA emphasized that it wanted to "reward companies that make healthier products." Meanwhile, the agency promised that it would increase enforcement action against companies "that appeal to consumers through false and misleading health claims."

As part of this new initiative, the FDA announced that it would consider both conventional foods and dietary supplements to be eligible for qualified health claims. Following the *Pearson v. Shalala* decision in 1999, the FDA had taken the view that qualified health claims would only be available for dietary supplements, because the case had been brought in relation to dietary supplement claims. However, because the decision was based on first amendment grounds, many stakeholders believed it should apply to all food products that were eligible for health claims; therefore, the FDA adopted the current view.

The FDA permitted four new qualified claims in 2003, relating to selenium and cancer, phosphatidylserine and cognitive dysfunction, nuts and heart disease, and walnuts and heart disease. All of these claims were evaluated on the basis of petitions submitted to the agency. The FDA suggested model language for each of the claims. The suggested language for the qualified claim for nuts was relatively positive, stating that "Scientific evidence suggests but does not prove that eating 1.5 ounces per day of most

Table 3
Qualified Health Claims Permitted by the US Food and Drug Administration as of September 2004

Qualified claims about cancer risk
Selenium and cancer
Antioxidant vitamins and cancer
Qualified claims about cardiovascular risk
Nuts and heart disease
Walnuts and heart disease
ω-3 fatty acids and coronary heart disease
B-vitamins and vascular disease
Qualified claims about cognitive function
Phosphatidylserine and cognitive dysfunction and dementia
Qualified claims about neural tube defects (NTDs)
Folic acid at a level of 0.8 mg, related to NTDs

nuts as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease." At the other end of the spectrum, the suggested model language for phosphatidylserine was very highly qualified. Table 3 provides a summary listing of the qualified health claims that are currently permitted by the FDA.

Another legal challenge was filed against FDA as a result of this newly expedited approach to evaluating qualified health claims. Two consumer advocacy groups, the Center for Science in the Public Interest and Public Citizen, charged that the new approach is illegal because it does not sufficiently protect consumers against potentially misleading information and in because the NLEA requires approval of health claims to go through formal rulemaking and not through informal procedures such as those the FDA used in dealing with the 2003 qualified health claims. However, the challenge was dismissed by the court *(42b)*.

All stakeholders agree that is it important for qualified health claims to be supported by a meaningful level of scientific evidence; however, defining the amount of evidence needed in each case will be an ongoing challenge for the agency.

8. STATEMENTS ABOUT EFFECTS ON STRUCTURE OR FUNCTION

The term "health claim," as used in this chapter, refers to health claims as defined in the NLEA of 1990—statements specifically describing the relationship between a food or food component and a disease or health-related condition, evaluated by the FDA and approved by regulation based on a finding that the claims are supported by significant scientific agreement. However, there are other types of statements relating to health effects of a product that can be made in labeling and advertising for both conventional foods and dietary supplements. The Food, Drug, and Cosmetic Act of 1938 distinguishes between drugs and foods largely on the basis of the uses for which these products are intended. Foods are simply products used for food. Drugs are defined partially as "articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals." Drugs also are defined as "articles (other than food) intended to affect the structure or any function of the body of man or other animals."

In the latter part of the drug definition, the law basically recognizes that foods have effects on structure and function and that such uses are not drug uses. The FDA has historically permitted some statements regarding the effects on structure and function to appear in food labeling and advertising, but it has argued in numerous legal cases that many particular statements about structure/function effects are actually implied disease claims and, therefore, not permissible for foods. The line between appropriate structure/function statements and illegal disease claims has been a subject of considerable discussion in the past decade because of new provisions that were added to the Food, Drug, and Cosmetic Act in 1994.

The Dietary Supplement Health and Education Act of 1994 (DSHEA) defines dietary supplements and permits them to bear "Statements of Nutritional Support." (The DSHEA also has numerous other provisions, which are not addressed in this chapter.) Dietary supplements have always been considered as a subcategory of foods, and they were classified as foods for special dietary use under the 1938 Food, Drug, and Cosmetic Act. The DSHEA reaffirms that dietary supplements are foods and provides a broad definition. A dietary supplement is defined as "a product (other than tobacco) intended to supplement the diet." Such products may contain vitamins, minerals, herbs or other botanicals, amino acids, or almost any other dietary substance that may be used to supplement the diet. Products may contain concentrates, metabolites, constituents, extracts, or any combination of the permitted types of ingredients. Dietary supplements may be in dosage forms, such as tablets or capsules, or may be in the physical form of conventional foods, provided they are not "represented for use as a conventional food" and are not intended as the sole item of a meal or of the diet.

Under the DSHEA, dietary supplement labeling is permitted to contain "Statements of Nutritional Support." These statements may describe the role of a nutrient or dietary ingredient that is intended to affect the structure or function of the body, may characterize the documented mechanism for such an effect, may relate to general well-being, or may claim a benefit related to a classical nutrient deficiency disease (provided the statement also discloses the prevalence of the deficiency in the United States). These statements may not claim to diagnose, mitigate, treat, cure, or prevent a specific disease or class of diseases.

Additionally. under the DSHEA, the manufacturer of any dietary supplement making a Statement of Nutritional Support is required to have substantiation that the statement is truthful and not misleading. Also, the label should contain the following disclaimer to avoid any implication that the statement is an FDA-authorized health claim or an FDA-approved drug claim: "This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease."

Marketers are required to notify the FDA within 30 d if they are making a Statement of Nutritional Support in labeling. According to a database compiled by AAC Consulting, the FDA has received letters of notification from hundreds of companies regarding more than 10,000 Statements of Nutritional Support *(43)*. The most commonly mentioned statements relate to emotional or mental health, cholesterol or the heart, energy and strength, immune function, antioxidant effects, joints and bones, digestion or gastrointestinal function, weight loss, urinary function or prostate health, and women's health. FDA does not evaluate or approve Statements of Nutritional Support, but it has responded to some letters of notification, indicating that the agency views the proposed statement as a disease claim or an unauthorized health claim.

Approximately 10% of the letters of notification have triggered FDA responses, which are called "courtesy letters" (to distinguish them from formal enforcement notices called "warning letters").

A few examples illustrate the current usage of Statements of Nutritional Support. In the area of immune function, Statements of Nutritional Support commonly appear on dietary supplements such as zinc or echinacea, indicating that the product supports a healthy immune system or enhances natural resistance. The FDA has found these statements to be permissible but has objected to any specific mention of diseases such as colds, flu, sore throat, and bronchitis. Statements about supporting normal joint function or joint flexibility are commonly used for products such as glucosamine and chondroitin sulfate. The FDA has found statements about supporting normal function to be acceptable but has objected to any specific reference to joint pain or to arthritis or rheumatism.

FDA regulatory oversight of Statements of Nutritional Support has thus far primarily concentrated on evaluating whether the statements legally qualify under the DSHEA and has not extended to probing the manufacturer's substantiation for the statements. Although many Statements of Nutritional Support clearly are consistent with the recognized effects of certain nutrients or botanical ingredients, there is substantial concern regarding the scientific basis of some label statements currently seen in the marketplace. Some groups have called for formal evaluation of the evidence supporting such statements. In its 1997 report, the Commission on Dietary Supplement Labels emphasized the importance of ensuring that Statements of Nutritional Support are adequately substantiated, so that consumers are provided with scientifically valid information *(44)*. The commission also suggested that summaries of the substantiation be made available to the public so that consumers and health professionals could better evaluate the claims being made.

Although it appears that Statements of Nutritional Support have, to some extent, accomplished the objective of better informing consumers about the uses of certain dietary supplements, there is concern regarding the blurring of traditional boundaries between permissible food claims and drug claims. In January 2000, the FDA finalized regulations that are intended to better define the boundaries between permissible structure/function statements and impermissible direct or implied disease claims *(45)*. In the final rule, the FDA provides numerous examples of permitted and prohibited claims to illustrate the agency's view of what constitutes an implied disease claim. Acceptable statements include claims relating to normal symptoms of the menstrual cycle or menopause (mild mood changes, cramps, hot flashes) and claims relating to mild memory problems or absentmindedness associated with aging. However, the agency finds that statements relating to benign prostatic hypertrophy would be considered disease claims, because the consequences are potentially more serious. Also, statements relating to lowering cholesterol or decreasing platelet aggregation would be considered to be implied claims about heart disease and, therefore, are not permitted. Statements about maintaining healthy function generally would be acceptable. One of the more surprising aspects of the final rule was the FDA determination that many approved claims for over-the-counter drug products are, in fact, statements about structure or function and not disease claims and that such statements may be used for dietary supplements as well. These include statements about preventing nausea from motion sickness, relieving occasional sleeplessness, and soothing stress or nervous tension.

There is an inherent tension between providing consumers with meaningful information about the health benefits of foods and dietary supplements and attempting to retain some legal or logical boundary to distinguish that information from the types of claims reserved for approved pharmaceutical products. With approved NLEA health claims that specifically claim to reduce the risk of serious chronic diseases and with DSHEA Statements of Nutritional Support that describe specific effects on the structure and function of the body, that tension is likely to continue into the foreseeable future. The food/drug distinction in the United States is not as sharp as it once was, and it may become even more blurred as policymakers seek to provide consumers with more information to enable them to more fully participate in efforts directed toward health promotion and disease prevention.

9. HEALTH CLAIMS POLICY OUTSIDE THE UNITED STATES

The policy debate regarding the dividing line between health claims made for foods and disease claims that should be restricted to drugs is a global phenomenon, based on scientific research into the physiological effects of foods and their resulting health benefits.

In Japan, only pharmaceutical products could traditionally be formulated and labeled for health uses. As a scientific consensus began to emerge regarding the importance of food choices in modifying the risk of chronic disease, the concept of functional foods began to develop in Japan *(46)*. In 1984, 1988, and 1992, special study groups on functional foods were appointed by the Ministry of Education, Science and Culture. Each group consisted of the top specialists in biochemistry, food chemistry, and agricultural chemistry and met for 2 to 3 yr to produce recommendations relating to the functional effects of foods in the modulation of physiological systems. In 1988, the Ministry of Health and Welfare established an Office of Health Policy on Newly Developed Foods, which studied how to best systematize the regulation of functional foods.

In 1991, the Nutrition Improvement Law officially established a category of products known as Food for Specified Health Uses (FOSHUs), which were eligible for health claims. FOSHUs are functional foods whose formulation and claims have been approved by the Minister of Health and Welfare based on clinical evidence that the product promotes health. Such foods cannot bear statements relating to the prevention or treatment of disease but may only bear claims regarding preserving or promoting health. Permitted claims included statements such as: "helps maintain a good gastrointestinal condition," "helpful for people concerned about high blood cholesterol level," and "suitable for people with mild hypertension." Such statements are considered to be sufficiently food-oriented to avoid conflict with the Pharmaceutical Affairs Law governing medicines in Japan.

FOSHUs that have been approved have included foods with functional ingredients, including oligosaccharides, lactobacillus, bifidobacteria, dietary fiber sources, peptides, sugar alcohols, and minerals. FOSHUs approved by the Ministry of Health and Welfare bear a graphic seal, and their labels must include information describing the functional effect, the amount to be used, and other directions for use. Contrary to health claims approval in the United States, the Japanese system does not generically confer FOSHU status for certain food ingredients or components. Rather, each individual product requires specific approval. As of September 2003, there were 371 FOSHUs approved in Japan *(47)*. Table 4 summarizes the approved products, categorized according to the type of claim.

Table 4
Foods for Specified Health Uses

Number of products	*Specified health use*	*Ingredients*	*Types of foods*
190	Helps maintain good gastro-intestinal condition	Oligosaccharides, lactobacillus, bifidobacterium, dietary fiber	Yogurt, beverages, table sugar, candy, vinegar, cookies, cornflakes, cereals
63	Good for those who have high serum cholesterol or triglycerides	Soy protein, peptides, dietary fiber, plant sterols/ stanol esters	Beverages, soy products, meat products, biscuits, cooking oils
41	Good for those who have high blood glucose	Dietary fiber, albumin polyphenols L-arabinose	Beverages
28	Good for those who have high blood pressure	Peptides, glucosides	Soft drinks, soup powder
26	Helps maintain strong and healthy teeth	Polyphenols, sugar alcohols	Candy, chocolate, chewing gum
23	Helps improve absorption of calcium or iron	Calcium citrate malate, casein phosphopeptide, oligosaccharides, heme iron	Soft drinks, tofu

Approved in Japan as of September 2003 *(47)*.

There is a striking dissimilarity between the types of health claims approved in the United States and the types of conditions for which FOSHUs are approved in Japan. In the United States, the majority of approved health claims relate to reducing the risk of cancer or heart disease. In Japan, half of the approved FOSHU claims relate to effects on the gastrointestinal tract, especially those relating to the nature of the microflora. In the United States, most foods for which health claims can be made are conventional foods that are major components of the diet (grains, fruits, vegetables), but some claims are permitted for fortified foods and dietary supplements. In Japan, many FOSHU claims are for specific food components delivered in the form of soft drinks or other beverages, but products in the form of tablets or capsules are also eligible.

In April 2001, the Japanese system for regulating foods and supplements with health claims was revised to add a category of "foods with nutrient function claims" *(46)*. In this category, statements about the recognized functions of vitamins and minerals are permitted, provided the nutrient is present within a prescribed range. For example, with vitamin A it may be stated that this nutrient "helps maintain vision at night" and "helps maintain healthy skin and mucosa." Also, the product must bear a disclaimer to distinguish these products from FOSHUs, and the disclaimer must state: "Unlike Foods for

Specified Health Uses, this food product has not been evaluated by the Ministry of Health, Labor, and Welfare."

In Canada, there has also been a long history of regulating all health claims as drug claims. The Canadian government has given consideration to the health claims approved in the United States and has concluded that some of the claims may be permissible in Canada, whereas others require additional review. In regulations published in January 2003, Canada finalized nutrition labeling regulations that also provide for health claims relating to calcium and osteoporosis, sodium and hypertension, dietary fat and heart disease, fruits and vegetables to reduce the risk of cancer, and sugar alcohols to reduce the risk of dental caries *(48)*. In a separate initiative, over the past 5 yr, Health Canada has been in the process of developing an entirely new regulatory system for "natural health products." In 1998, the Standing Committee on Health produced a report in which 53 recommendations were proposed as the basis for future regulation of natural health products. In 1999, the Natural Health Products Directorate was established within Health Canada to develop a comprehensive framework, with extensive stakeholder input, and to administer the system. The final natural health products regulations were published in the Canada Gazette, Part II, on June 18, 2003, and became effective in January 2004 *(49)*. A broad range of products are covered, including vitamins, minerals, herbal remedies, traditional medicines (such as Traditional Chinese Medicine), homeopathic medications, probiotics, amino acids, and essential fatty acids. Most natural health products are considered as a subcategory of "drugs," and health claims are permitted, provided that the claims are in accord with the findings of a review process that covers safety as well as efficacy. Monographs are prepared for a large number of ingredients or products to facilitate the ability to make claims that are recognized as having appropriate scientific support. Natural health products must be manufactured under Good Manufacturing Practices (as defined in the regulations), the producers must have site licenses, and products will eventually be required to bear a Natural Product Number (comparable to the current Drug Identification Number). Observers worldwide are watching the Canadian regulatory approach with great interest, because it appears to offer some unique opportunities to meet consumer demands for health-related products yet maintains a high level of control over product safety and quality as well as validity of claims.

In Europe, health-related claims currently are permitted in most countries only for drug products, although there are provisions under which some claims may be specifically licensed by a national government for use with foods or food ingredients. In July 2003, the European Commission proposed a new directive regarding health claims for foods, which, if adopted, ultimately would require member governments to bring their national policies into line with the directive *(50,51)*. The proposed directive would apply to health claims made in advertising as well as labeling and would permit only claims supported by "generally accepted scientific data." A health claim is defined as a claim that "states, suggests or implies that a relationship exists between a food category, a food, or one of its constituents and health." The proposal includes a unique provision that provides a period of exclusivity to submitters of proprietary data supporting a health claim, so long as the claim could not have been approved without submission of the proprietary data. Structure/function claims would also be permitted, and if the Directive is adopted, a positive list of well-recognized functional claims is to be developed within 3 yr of implementation. Under this proposal, health claims would need to

be reviewed by the European Food Safety Authority and approved by the European Union Commission, and a register of health claims would be established. The proposed directive requires the approval of the European Parliament and the Council of Ministers before it can become final.

The Codex Alimentarius is an international body that develops standards and policies that are applicable to foods marketed worldwide. A policy recommendation relating to health claims has been debated by the Codex Committee on Food Labeling over a period of several years *(52)*. The committee's current recommendation relates to nutrition claims as well as health claims and asserts that these claims "should be consistent with national health policy, including nutrition policy, and support such policies where applicable." The committee recommends that such claims be permitted in food labeling, provided that they are "based on current relevant scientific substantiation and the level of proof must be sufficient to substantiate the type of claimed effect and the relationship to health as recognized by generally accepted scientific review of the data and the scientific substantiation should be reviewed as new knowledge becomes available." This recommendation was forwarded to the full Codex Alimentarius Commission for adoption in 2003, but it was returned to the committee for further consideration regarding whether the policy should apply only to food labeling or whether it should also apply to food advertising. Codex recommendations are particularly significant, because many national governments develop or modify their food labeling policies based on Codex models.

10. OUTLOOK FOR THE NEXT DECADE

There is a worldwide trend to reconsider national and international policies that have traditionally restricted health claims for foods. Scientific evidence supports the concept that dietary patterns, food choices, and the intake of specific food components can affect the health of individuals and ultimately can have an impact on public health. It seems likely that statements about the health benefits of foods will become a more prominent component of food labeling and advertising in many parts of the world. This phenomenon is already well-advanced in the United States and is moving forward under various frameworks in other countries and regions of the world. It remains to be seen whether divergent national and international policies will eventually be harmonized in the interest of facilitating the global marketing of foods with health benefits.

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36 Teaching Preventive Nutrition in Medical Schools

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KEY POINTS

- On a daily basis, most physicians need specific knowledge and skills regarding nutrition to provide optimal care for their patients.
- Medical organizations and nutrition educational task forces agree that medical students need to learn about all clinically relevant nutrition topics.
- Comprehensive nutrition education outlines the impact of dietary exposure on disease development and teaches practical diagnostic and intervention skills.
- Computer-assisted nutrition instruction is effective and ensures systematic coverage of the full range of knowledge and skills.
- Adaptive instruction can tailor content presentation to student needs and save time by avoiding unnecessary repetition.

1. WHY SHOULD HEALTH PROFESSIONALS HAVE NUTRITION EXPERTISE?

Insufficient, excessive, or imbalanced availability of macro- and micronutrients is a major contributing cause of morbidity and mortality worldwide. As people age, the patterns of nutritional intake play a significant role in the onset and progression of diseases: 5 of the 10 leading causes of death have a strong relationship with poor diet *(1,2)*. Obesity is epidemic in the United States, contributes to health risk, and currently contributes $92 billion (more than $700 per obese person) to health care costs *(3)*. In many instances, particularly in affluent countries such as the United States, well-targeted dietary changes in patients that are encouraged by health professionals can significantly change disease risk and prevent or delay morbidity and mortality.

The early successes of nutrition interventions virtually eradicated simple deficiency diseases such as scurvy, pellagra, and rickets in developed countries. The need has shifted to more complex nutrition patterns that are difficult to change and that influence health outcomes over the long-term. The growing obesity epidemic drives a secondary wave of

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diabetes, cardiovascular disease (CVD), renal failure, and cancer. The prevalent pattern of overconsumption causes additional specific health risks that are independent of the obvious mismatch of energy expenditure and intake. Therefore, the excessive insulin release triggered by high intake of rapidly absorbed carbohydrates promotes some common cancers, and the hyperlipidemias related to high intake of saturated fats and partially hydrogenated fats (*trans* fats) accelerate atherosclerotic damage. Paradoxically, even with a plentiful food supply, most people do not get enough of all beneficial nutrients and other food constituents to ensure optimal health and minimize lifetime risk from disease. For example, most obese people could likely lower their risk for CVD, cancer, and other disease if they acquired enough folate from food or other sources. Understanding these complex nutritional interactions requires grounding in nutrition sciences.

Physicians and other health professionals need to be intimately familiar with nutrition–disease relationships, just as they are with other disease-causing agents and conditions. They are in a position to work with their patients on preventive measures before nutrition-related diseases cause harm that is difficult to correct or irreparable. Vaccinations, well-visits, visits for minor illnesses or injuries, and sports physicals provide opportunities to discuss the risk of diabetes with the parents of obese children and young adolescents and to direct them to appropriate resources. Nutrition information provided during routine gynecological examinations can help women to lower their risk of CVD and reduce the risk of birth defects in the event of a future pregnancy. Neurologists need to know about the consumption of some critical foods and dietary supplements in detail to optimize medications for a patient with epilepsy. Full attention to feeding arrangements and nutritional status of elderly patients during hospital rounds are crucial for the rapid and sustained recovery after surgical interventions. These are just a few examples to indicate how commonly nutrition intersects with the daily practice of virtually all clinical specialties.

Physicians also have unrivaled authority on health issues. The private and public discourses of physicians can have significant impact on individual behaviors and often influence policy decisions. For all these reasons, it is imperative that physicians and health professionals are knowledgeable regarding nutritional issues.

2. WHAT NUTRITION KNOWLEDGE HELPS HEALTH PROFESSIONALS TO PROMOTE DISEASE PREVENTION?

Health professionals need a general framework of nutrition knowledge and practical skills to be effective. Similarly to other areas of medicine, new insights develop rapidly and occur constantly; therefore the standards of practice evolve continuously. Nutrition issues pose a particular challenge, because the public is so familiar with eating and is constantly inundated with a mixture of credible, irrelevant, and outright false information regarding nutrition. Patients ask questions about everything from weight loss to dietary supplements. Physicians should be able to provide specific and reliable guidance within the time constraints of typical consultations. They perform best when they can draw on a solid understanding of nutritional theory and possess frequent updates on current practice from credible sources (not simply from the mass media).

Recent medical graduates usually do well at the diagnosis and pharmacological treatment of metabolic diseases such as diabetes and hyperlipidemias, because these are topics of instruction in all medical school curricula. Coverage of preventive nutrition

tends to be much more limited and largely depends on the interests of particular instructors. Several organizations have suggested a set of nutrition topics that medical school curricula should cover *(4,5)*. The most recent set of proposed topics was initiated by the American Medical Student Association's Nutrition Committee and was endorsed by a panel of experienced nutrition educators *(5)*. The 92 listed topics were of varying complexity and sometimes covered entire subdisciplines (e.g., "pregnancy"). Some did not have a clear correlate in current nutritional practice (e.g., "schizophrenia"), and others may have more properly been the domains of other disciplines ("peptic ulcer disease"). Several important nutrition issues were not included. The competencies corresponding to the amended and consolidated list (Table 1) require many additional hours of study and practice than most medical schools can currently allocate. Similarly, the Curriculum Committee of the Nutrition Academic Award Program defined detailed nutrition knowledge objectives for both medical students and more advanced learners *(6)*.

3. WHERE AND WHEN CAN PHYSICIANS LEARN TO USE PREVENTIVE NUTRITION?

Practically every US medical school attempts to offer some type of nutrition education. In response to one of our surveys *(7)*, only 1 out of the 95 schools that provided detailed information did not teach nutrition to their medical students. In most (90%) of the responding schools, nutrition education was a required part of the curriculum. A comprehensive survey of the American Medical Association Liaison Committee on Medical Education (LCME) produced similar results *(8)*. Nonetheless, graduation exit surveys persistently find that students in most medical schools are greatly dissatisfied with nutrition education *(9)*. This is not be surprising, because very little time is actually devoted to teaching core concepts of nutrition. In our survey, we found that even among the schools that reported a nutrition requirement, the majority (70%) offered fewer than the minimum 25 h that is considered necessary by the Food and Nutrition Board of the National Academy of Sciences *(10)*.

These shortfalls may be partly related to lack of visibility in most medical schools: only 31 of the 126 LCME-accredited medical schools in the United States list a department, division, or specialty in which the title makes any reference to nutrition. Currently, only eight medical school departments or divisions are specifically dedicated to nutrition. The other 23 medical schools list nutrition as part of the organizational title (usually secondary to gastroenterology or endocrinology) of divisions in medicine or pediatrics. A recent survey of medical school faculty also did not list nutrition as a separate discipline *(11)*. Additionally, the LCME requirements for medical school accreditation do not even mention nutrition education *(12)*. Of course, the challenge exists in that the constantly increasing curriculum load of medical students leaves little room for additional content *(13)* and that the more visible disciplines will compete more successfully for course time. The political structure of medical schools is such that the curriculum for medical students, in part, defines the territory for scientific disciplines in medicine. For nutrition to succeed in the medical school environment, it must be included as a core part of student training *(14)*. Because nutrition is not included as a core part of student training, a message is sent to future physicians that this discipline is not important.

Table 1
Expected Nutrition Competencies for Medical Graduates

Basic nutrition
Energy (sources, deficiency, calculations)
Physiology of hunger and satiety
Physiology of thirst
Protein (sources, bioavailability, deficiency)
Carbohydrates (simple and complex, requirements)
Fats (ω-3, ω-6, saturated, trans, effects)
Dietary fiber (types, sources, effects)
Vitamin A (sources, deficiency, excess)
Antioxidants (types, sources, effects)
Vitamin C (sources, actions, deficiency)
Vitamin D (skin synthesis, dietary sources, deficiency)
Vitamin K (sources, deficiency, anticoagulation)
Thiamin (sources, deficiency)
Riboflavin (sources, deficiency)
Niacin (sources, deficiency)
Folate (sources, deficiency)
Vitamin B_{12} (sources, deficiency)
Water and electrolytes (Na, K, Cl)
Calcium and phosphate (sources, deficiency, excess)
Iron (sources, bioavailability, deficiency, excess)
Zinc (deficiency)
Trace minerals (I, Cu, Cr, Mn, Mb, Se)
Nutrition and immunity

Nutrition Assessment
Diet history taking
Nutrition physical examination (body composition, skin)
Laboratory evaluation
Assessment of energy balance
Assessment of protein intake
Assessment of carbohydrate intake
Assessment of fat intake
Assessment of vitamin intake
Assessment of antioxidant intake
Assessments of electrolyte intake and balance
Assessments of mineral and trace element intake
Assessment of fiber intake

Preventive Nutrition
Conception and pregnancy
Lactation
Growth and development
Second half of life (cognitive decline, osteoporosis)
Oral health
Cardiovascular disease
Cancer
Obesity
Hypertension
Criteria for an adequate diet

(Continued)

Table 1 *(Continued)*

Nutrient intake recommendations
National nutritional programs and goals
Vegetarianism
Dietary Treatment of Disease
Eating disorders
Failure to thrive
Nutritional anemias
Diabetes
Cancer
Hyperlipidemia
Renal failure
Diarrhea
Alcohol abuse and liver disease
Celiac disease
Kidney stones
Hyperuricemia and gout
Hospital malnutrition
Surgery, trauma, and infection
Food-borne illness
Drug–nutrient and supplement–drug interactions
Primary malnutrition
Food intolerances and allergies
Inborn errors of metabolism
Acquired immunodeficiency syndrome
Nutritional Intervention
The "MD-RD" team
Nutritional supplements
Enteral nutrition support
Parenteral nutrition support
Writing nutrition prescriptions
Writing nutrition referrals
Cultural issues

4. HOW CAN NUTRITION BE TAUGHT?

These difficulties can be overcome, as demonstrated by several institutions. Among the respondents to our survey *(7)*, 25 schools reported that nutrition education was integrated into a larger part of the curriculum, sometimes across several years. Since then, anecdotal information from some institutions indicates that this type of instructional arrangement is on the rise. The establishment of an integrated curriculum with comprehensive nutritional content improved students' performance and boosted satisfaction *(9)*. The cost was allocation of an additional 40 h of nutrition instruction according to a topic list similar to the one mentioned earlier. In our survey, only three other institutions reported a similar allocation of curricular time to nutrition education. An integrated case-based approach at another medical school also virtually eliminated student dissatisfaction, although education efficacy was not reported *(15)*. Again, a comprehensive list of nutrition competencies was added to the teaching objectives of most basic and clinical courses. The disadvantage of such fully integrated curricula is that students are

less likely to appreciate the full potential of nutritional assessment and intervention because they do not see nutrition as a fully developed discipline. Usually, many of the instructors, who are tasked with teaching nutrition as part of another discipline, are not fully versed in all areas of nutrition. For example, although some instructors may be highly qualified to present certain aspects of nutrient metabolism from a basic science perspective, they may not be able to convey the relevance of clinical interventions. Careful curriculum development and close interaction between nutrition educators and instructors from other disciplines is essential for the success of integrated curricula *(9,15)*. Instructors at the University of Pennsylvania have developed materials for case-based instruction, which are collected in the textbook *Medical Nutrition and Disease (16)*. Case-based learning integrates basic science and clinical practice principles in an accessible format that is especially suitable for small-group discussions and as a starting point for self-study. Introductory nutrition sessions with the students and the labeling of nutrition-specific content as a discrete entity fostered an identity for the nutrition component and met students' high expectations of nutrition education.

We must recognize the trend among medical schools to increase curriculum content that focuses on problem-solving in small group teaching settings *(17)*, whereas structured lecture time is being reduced. New materials should be introduced with innovative approaches that require a minimum of added contact hours *(18)*. Computer-based instruction offers an alternative, particularly for institutions that have difficulty covering the full range of nutrition education. Previous experience supports the view that medical students learn well with computer-based instruction *(19,20)*. Students can learn at their own pace and at a time that they choose. The interactive format can ensure that material is well-retained and identifies knowledge gaps through self-assessment features. Slow and difficult navigation through materials caused problems with earlier applications, because the user could not easily view adjacent pages or quickly browse through a chapter. However, computers have become much faster, program interfaces have become more sophisticated, and users have grown more adapted to extended work with learning modules. Earlier barriers to computer-assisted instruction have lost importance, because medical students now routinely use electronic learning resources, and laptop computers have become a constant presence even in lecture halls.

We have developed a computer-taught modular nutrition curriculum that covers most of the topics listed in Table 1 and takes 20 to 30 h to complete *(21,22)* (Fig. 1). The aim of this curriculum is to (a) foster a thorough understanding of nutrient sources, metabolism, and functions; (b) lead to an appreciation of nutritional lifestyle for disease development and amelioration; (c) teach specific diagnostic and interventional skills; and (d) demonstrate the collaboration of physicians with dietitians and other health care professionals. Video case presentations provide a framework for the application of basic science concepts and practical clinical skills. Clinical patient information becomes accessible as the user makes diagnostic and treatment decisions and the case evolves. Knowledge items are taught and reinforced with a variety of methods, including animations, case-based interactions, and short quizzes. The wide range of optional resources includes an extensive glossary, detailed intake recommendations, video-based demonstration of anthropometric techniques, and extensive information regarding diagnostic tests. Review copies of the programs are available to medical schools at no charge (http://www.med.unc.edu/nutr/nim/). In 2003, 87 (69%) of the 126 accredited US medical

Fig. 1. Sample screens from Nutrition in Medicine CD-ROM series.

schools used one or more of the programs. Sixty-three international medical schools used the CD-ROM series.

The content covers sources, metabolism, and pathological consequences for all macronutrients and most micronutrients in a clinical context. The computer-based presentation provides an opportunity to ensure comprehensive coverage of all topics without strict adherence to a systematic sequence. Therefore, vitamins are integrated throughout the modules rather than in a section of their own. This brings the basic nutrient information closer to the clinical context and makes it more likely to be drawn upon as typical clinical situations arise later in practice.

An important element of the instruction concerns how to conduct dietary interviews and avoid typical pitfalls. The assessment of nutrient intakes (as listed among the essential topics) is an example of specific diagnostic skills conveyed in the programs. Because physicians in clinical practice rarely have the time for a complete dietary history (which is also a topic of instruction), the program draws the learner's attention to the most important food sources. We developed a series of simple questions to identify US patients that are most likely to be at risk for particular nutrient deficiencies or imbalances (Table 2). When the particular nutrients are discussed, images of the dominant sources are displayed with the questions and commentary on typical at-risk populations. At a later time, interactive exercises reinforce the use of these assessment questions. Emphasis is placed on taking a dietary supplement history as an indispensable part of some office visits. The students are first taught the main considerations for obtaining a complete

Table 2
Questions for the Identification of Patients Most Likely at Risk of Individual Micronutrient Inadequacies

How many servings of grains (bread, cereal, pasta, rice, corn) and pork do you consume per day?
Thiamin alarm threshold: Has fewer than three servings of grains or one serving of pork per day. People who severely restrict their carbohydrate or total energy intake and do not eat pork on a regular basis are most likely to have low thiamin intake.
How many servings of grains, milk, and meats do you have per day?
Riboflavin alarm threshold: Has fewer than three servings of grains, milk, and meats per day. People with severely restricted intake, such as the elderly and people on very low calorie diets, are most likely to have low intake.
How many servings of potato, sweet potato, corn, meat, poultry, fish, banana, breakfast cereal, and watermelon do you have per day?
Vitamin B_6 alarm threshold: Has fewer than three servings of potato, sweet potato, corn, meat, poultry, fish, banana, breakfast cereal, and watermelon per day. Look for people who eat little overall.
How many servings of breakfast cereals, dark-green vegetables, legumes, orange and orange juice do you have per day?
Folate alarm threshold: Has fewer than three servings of breakfast cereals, dark-green vegetables, legumes, and orange per day. Look for people who do not like greens and legumes.
How many servings of seafood, meat, dairy products, and eggs do you have per day?
Vitamin B_{12} alarm threshold: Has less than one serving of seafood, meat, dairy products, and eggs per day. Look for people who avoid animal-derived foods.
How many servings of fruits and nonstarchy vegetables do you have per day?
Vitamin C alarm threshold: Has less than one serving of fruit or nonstarchy vegetable per day. Look for people who eat few fruits and vegetables.
Do you eat mostly fat-free foods?
Vitamin E alarm threshold: Avoids oils, fats, nuts, and seeds. Look for people who eat mostly fat-free foods and avoid added fat.
How many servings per day of fruits and nonstarchy vegetables with colored flesh do you have per day?
Carotenoids alarm threshold: Has less than one serving of fruits and vegetables with colored flesh per day. Look for people who eat few colored fruits and nonstarchy vegetables.
How many servings of milk, calcium-fortified juice, yogurt, and cheese do you have per day?
Calcium alarm threshold: Has fewer than three servings of milk, calcium-fortified juice, yogurt, and cheese per day. Look for people who avoid milk and calcium-fortified juices, do not get enough vitamin D, and consume too much phosphate.
How many servings of milk per day and fish per week do you consume?
Vitamin D alarm threshold: Has less than one serving of milk per day or fish per week. Look for people who avoid the sun, do not eat fish, nor drink fortified milk.
How many servings of meat, processed cheese, and cola do you have per day?
Phosphate alarm threshold: Has more than five servings of meat, processed cheese, and cola per day. Look for people who frequently have cola or cheeseburgers.

supplement history. They can then use a video case format to interactively explore the issues by prompting the interviewing physician to question her patient. This interaction demonstrates how supplement usage often only arises in response to specific questioning. Focus group testing demonstrated that just a few minutes of exposure provided very good working skills that can be used directly with patients.

5. AN ADAPTIVE LEARNING MODE

Identifying existing nutrition instruction in courses of biochemistry, physiology, pathology, endocrinology, gastroenterology, and other disciplines is helpful but will be insufficient without considerable expansion. Introducing a comprehensive stand-alone nutrition curriculum would be desirable but likely requires a minimum of 25 h of instruction *(10)*. Evidently, without a significant restructuring, few schools would be able to present their existing curricula and add several more hours of nutrition education. Therefore, the best hope for adding nutrition content is the use of more efficient and targeted instruction.

It is worth noting that nutrition properly deals with the metabolic and health consequences that result from different levels of intakes. To establish a legitimate territory for nutrition in medical schools, time teaching nutrition in the core curriculum must be spent wisely. Content must be presented so that it becomes clear that nutrition uses evidence-based approaches and teaches components that are critical, as well as familiar, to the entrenched medical disciplines.

Although nutrition is a highly important topic of study, there is a valid argument that the existing medical curriculum is overburdened and struggling to fit within a 4-yr degree program. Thus, nutrition instruction should build on prior courses of anatomy, biochemistry, and physiology and presuppose basic understanding of major disease processes. Although there are considerable overlaps, there is much nutrition-specific content that should be favored for inclusion at the expense of basic science or general clinical material. Thus, we found that by the second half of our students' second year (prior to any specific nutrition instruction), they had adequate knowledge regarding treatment of diabetes but knew little about nutritional prevention of cancer or dietary support after diagnosis. Even within individual modules, as is true for traditional lectures, some of the material duplicates earlier presentations in other courses. There would be a considerable amount of time saved if it were possible to focus only on those knowledge items that a particular class or even a particular individual was not familiar with.

We explored the viability of such adaptive instruction in a class of second-year medical students *(19)*. All 117 students in a class took a test with multiple-choice questions covering the main topic areas of our cancer instructional program. Half of the students then used a shortened program version that omitted the topics that most in the class were already familiar with. The other students used the full version. Tailoring the instruction based on students' prior knowledge cut the time requirement to about half and achieved nearly the same instructional efficacy. We provide a mechanism whereby all of our current programs can be adapted by instructors to meet student needs by following the instructor's own preferences, known overlaps with prior courses, or based on tests as outlined earlier (Nutrition in Medicine Lite; http://www.med.unc.edu/nutr/nim/). We are currently engaged in the development of a Web-based/CD ROM hybrid system that can collate individual instructional items across the entire curriculum based on instructor preference and prior student knowledge.

6. CONCLUSIONS AND RECOMMENDATIONS

Nutrition education is an indispensable curriculum component that prepares future physicians for daily health care challenges. Theoretical knowledge and practical skills help future physicians to cope with ever-expanding nutrition information and can make nutrition assessment and effective intervention an integral part of daily practice. The time period of undergraduate medical education constitutes the formative years that greatly determine the attitudes, knowledge framework, and basic skill sets of future physicians. During this critical time, medical students should be offered a comprehensive curriculum that focuses on practically relevant nutrition topics and visibly demonstrates the importance of in-depth nutrition knowledge for effective health care. Given the increasing importance of diet-related diseases, most medical schools need to allocate a significantly greater amount of time to specific nutrition education. Computer-based instruction can be an effective resource to augment the efforts of a limited number of nutrition instructors and can ensure a sufficient scope of teaching and training. Because the programs can be adapted to the particular needs of each medical school and even of individual students, the gains from more efficient delivery may be a convincing argument for course directors and curriculum committees trying to fit ever more content into an already overburdened curriculum.

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37 Preventive Nutrition Throughout the Life Cycle

A Cost-Effective Approach to Improved Health

Adrianne Bendich
and Richard J. Deckelbaum

KEY POINTS

- More than 60% of adults in the United States self-report that they are in excellent health, although more than 60% are overweight or obese and almost 60% do not participate in any vigorous physical activity. Obesity is a growing problem globally.
- A major finding in the 20th century was that a simple B vitamin (folic acid) could prevent a very serious birth defect of the neural tube; furthermore, folic acid-containing multivitamins taken during the periconceptional period significantly reduced cardiovascular and renal defects as well as neural tube and cleft lip–cleft palate defects.
- Low intakes of many of the essential micronutrients are associated with increased risk of low birth weight and/or premature births, maternal morbidity and mortality in both developed and undeveloped nations. Interventions have the potential to reduce health care costs by billions of dollars annually.
- Childhood undernutrition, also seen in both developed and undeveloped nations, significantly reduces intellectual capacity and increases infection-related morbidity/mortality. Micronutrient fortification is predicted to be highly cost-effective.
- Regarding adult chronic diseases/conditions, 5 of the 10 leading causes of death are related to diet. Although recent intervention trials with single or multiple micronutrients did not show decreases in secondary events, baseline findings continue to confirm the strong associations between higher than average intakes and significantly reduced risks of, for example, cancer and cardiovascular disease.
- Osteoporosis, cataracts, age-related macular degeneration, diabetes, and hypertension appear to be likely candidates for preventive nutrition intervention strategies. Predicted annual cost savings above and beyond the costs for the nutritional interventions could reach $50 billion.

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1. INTRODUCTION

The most recent data from the US Centers for Disease Control indicated that "in 2001, 64% of adults 18 years of age and over reported excellent or very good health." However, 58% of adults never participated in any type of vigorous leisure-time physical activity, and 13% of adults did not have a usual place of health care. Twelve percent of adults had been told by a doctor or other health professional that they had heart disease and 21% had been told on two or more visits that they had hypertension. Nearly one-fourth of adults were current smokers, and 22% were former smokers. Moreover, based on adults ages 20 yr and older with a body mass index greater than 25, 64% were overweight or obese and 30% were frankly obese *(1)*.

Within the past 5 yr, there has been a growing appreciation of the value of nutritional interventions as cost-effective and safe means of maintaining health and decreasing risk of major chronic diseases. Moreover, an increasing body of evidence suggests that there are more commonalities than differences among the nutrition recommendations to reduce risk factors for diseases; for instance, both cancer and cardiovascular disease (CVD) prevention recommendations include increased consumption of fruits and vegetables *(2–11)*. Similarly, increased whole grain intake (as compared to refined grains) has been associated with decreased prevalence of CVD, cancer, and type 2 diabetes *(12–17)*. A simple dietary change (increasing the number of servings of low-fat, calcium-rich dairy foods by two each day) has significant potential for an annual $26 billion in health care savings in the costs associated with hypertension, obesity, type 2 diabetes, coronary artery disease (CAD), stroke, osteoporosis, kidney stones, colorectal cancer, and pregnancy-associated pre-eclampsia *(18)*. Obesity is becoming the leading cause for concern because of the multifold attendant morbidities. Chapter 15 by Frier and Greene is especially informative *(19)*. Moreover, the unexpected large increase in childhood obesity and excessive weight, reviewed extensively by the leading researchers in Chapters 12–14 in this volume *(20–22)*, outline the unfortunate consequences not only in childhood but also in adulthood, even if the obese or overweight child develops into a normal-weight adult. Obesity is linked to between 280,000 and 325,000 deaths per year in the United States and is more costly than smoking-related deaths (the number one killer). It is estimated that obesity-related expenditures reached $75 billion in 2003. Currently, about 27% of US adults and children are classified as obese *(23)*. Other nations are also calculating the health care costs associated with obesity. The pharmacoeconomics of managing childhood and adolescent obesity was recently reviewed by Kiess et al. *(24)* in a population of German children. The authors reported that a cross-sectional study of more than 2500 children in Leipzig found that 29% of those ages 7 to 18 yr were overweight and 16% were obese. Obesity is a growing global problem—not only a US problem.

Decreased fat intake, particularly saturated fat intake, is clearly linked to decreased serum cholesterol levels and decreased prevalence of cardiovascular complications such as CAD *(18)*. Although recent studies place some doubt on the contribution of fat intake toward increasing risk of breast cancer, high levels of fat intake are still associated with the risk of some cancers and type II diabetes *(1,25–27)*. Foods that are rich in other dietary components, including fiber, complex carbohydrates, and micronutrients, appear to decrease the risk of certain forms of cancer as well as coronary heart disease (CHD) and manifestations of diabetes *(19,20,28,29)*. A recent analysis of the direct medical costs of care for diabetes, based on actual cases, showed that the lowest costs

were associated with those diabetics who were diet-controlled. Obesity added significantly to health costs, as did use of insulin and more advanced disease resulting in kidney failure and need for dialysis. Dialysis alone increased costs 11-fold *(30)*.

The major chronic diseases also share a number of common cellular and biochemical mechanisms in their pathogenesis *(1,23,31)*. For example, cell proliferation is common in both atherosclerosis and cancer and is of importance to some of the complications associated with diabetes. Changes in signal transduction and gene expression relate to cancer, atherosclerosis, obesity, and diabetes. Additionally, DNA modifications likely contribute mechanistically to each of these disease classes. Importantly, in examining the sets of nutrition recommendations that are aimed at reducing the risk of several chronic diseases and that are developed by a number of different private and government organizations, the different recommendations show far more common themes and commonalities than differences. The recommendations of most nutrition and public health groups are very similar to the 2000 United States Dietary Guidelines of the US Department of Health and Human Services and the US Department of Agriculture, which include the "ABC" recommendations outlined in Table 1 *(16)*.

One important issue deals with how to implement the current dietary recommendations for all population groups regardless of their economic and educational status. Where will food fortification and micronutrient supplementation be of most benefit? The reality is that the majority of populations, both in the United States and elsewhere, do not follow dietary recommendations *(12,32–34)*. Given the available evidence that higher fruit and vegetable as well as whole grain intakes can have marked effects on reduction of chronic disease risk, it is quite likely that improved diets alone would be a major step forward in chronic disease risk reduction and economic cost. However, it is also clear that fortification and supplementation of certain nutrients has been associated with reduced risk of chronic diseases in populations that have not routinely consumed the highest intakes of recommended foods *(35–37)*. Therefore, an inclusive approach that combines the best of many nutritional delivery systems and includes physical activity and smoking reduction may provide a greater potential for cost-effective measures to reduce disease.

For developing countries, a new report in 2004 from the United Nations Children's Fund (UNICEF) and the Micronutrient Initiative *(38)* found that lack of basic vitamins and minerals in the diet holds back economic development in virtually every country in the southern hemisphere. A lack of key vitamins and minerals is responsible for impairing intellectual development, compromising immune systems, and increasing the risk of serious birth defects. The report found that fortification of wheat flour with iron and folic acid in the 75 most needy countries was expected to reduce iron deficiency by 10%, and birth defects could be lowered by one-third. Such fortification would cost a total of about $85 million, which is about $0.04 per person. The estimated result is that these countries would gain $275 million in increased productivity and $200 million from enhanced earning potential.

1.1. Objectives

The major objective of this chapter is to examine the wealth of data presented in this volume, in the more than 50 chapters in the two earlier editions of *Preventive Nutrition (39,40)*, in relevant chapters from *Primary and Secondary Preventive Nutrition (41)*, and in other published studies and to look at the potential cost savings if these strategies

Table 1
Aim, Build, Choose—for Good Health

Aim for fitness
Aim for healthy weight.
Be physically active each day.
Build a healthy base
Let the Food Pyramid guide your food choices.
Choose a variety of grains daily—especially whole grains.
Choose a variety of fruits and vegetables daily.
Keep food safe to eat.
Choose sensibly
Choose a diet that is low in saturated fat and cholesterol and moderate in total fat.
Choose beverages and foods that limit your intake of sugars.
Choose and prepare foods with less salt.
If you drink alcoholic beverages, do so in moderation.

were adopted. We highlight those nutritionally related interventions that can improve health, reduce disease risk, and save on health care costs. Therefore, our goal is to document the totality and the consistency of the data and then attempt to quantify the savings that could exist once preventive nutrition strategies are instituted.

Each section includes data related to the preventive nutrition interventions at the level of individuals and populations and then discusses the health economics potential. The discussion follows the life span and thus begins with the anticipation of conception and concludes with an examination of the diseases/conditions that most affect seniors. Emphases are placed on the role of nutrition in the prevention of birth defects and premature birth and the cost-effectiveness of nutritional interventions for CVD, cancer, and osteoporosis prevention. The information regarding health economics is based mainly on US national databases of hospital costs. Globally, there will be many differences in the economic value of nutrition interventions, and these obviously also differ between countries at various stages of economic development and availability of medical care.

2. NUTRITIONAL INTERVENTIONS TO OPTIMIZE PREGNANCY OUTCOMES

2.1. Birth Defects

When *Preventive Nutrition* was published in 1997, the objectives of the researchers who wrote the chapters involved with optimizing pregnancy outcomes were to reduce the risk of birth defects *(42)* and premature birth *(43)* so that the infant morbidity and mortality would be decreased. Secondarily, there were also strategies that would reduce maternal morbidity and mortality and reduce the potential for paternal sperm DNA damage *(44)* and, consequently, also improve neonatal health prospects. The data primarily came from studies in developed countries where frank nutrient deficiencies are rare. Therefore, the dietary and/or supplementation strategies were implemented in relatively well-nourished women. The Barker Hypothesis that chronic diseases of middle age begin *in utero* further heightens the need to enhance the nutritional status of women of childbearing potential. Improvement in birth outcomes, especially decreasing prevalence of low birth weight, may also improve the health of newborns when they reach

middle age and older *(45–48)*. Unfortunately, current economic predictions cannot account for the potential reductions in health care costs that would be realized in the next half-century or more. Nevertheless, the benefits of optimizing pregnancy outcomes to improve current neonatal and maternal health are more than sufficient to justify the nutritional interventions discussed here.

The recent UNICEF report indicates that developing countries are examining the economic burden of adverse pregnancy outcomes *(38)*. Iodine deficiency, prevalent in many African and Asian nations, has serious and irreparable consequences in pregnancy. As many as 20 million babies every year are born mentally impaired as a result of maternal iodine deficiency. Severe iron deficiency anemia contributes to the deaths of an estimated 50,000 women a year during childbirth. Folate deficiency is causing approx 200,000 severe birth defects annually and is associated with roughly 1 in 10 adult deaths from heart disease. Food fortification with essential vitamins and minerals in foods that are regularly consumed by most people (such as flour, salt, sugar, cooking oil, and margarine) is the preferred method to increase consumption in the developing countries and is predicted to cost only a few cents per person per year. Supplementation is considered for only the most vulnerable groups (particularly children and women of childbearing age), using vitamin and mineral supplements in the form of tablets, capsules, and syrups. Although the actual costs of the supplements are low, implementation costs are higher than for fortification. However, fortification and supplementation programs cannot be successful without a parallel program to control diseases such as malaria, diarrhea, and parasitic infections, which reduce the ability of the body to absorb and retain essential vitamins and minerals. Nevertheless, it is estimated that in the 75 most needy countries, if wheat flour was fortified with iron and folic acid, iron deficiency could be reduced by 10% and birth defects could be lowered by one-third.

Birth defects are the leading cause of infant mortality and morbidity in the United States. Cardiovascular birth defects are the most common birth defect; neural tube birth defects (NTDs) are the second leading defect. One major preventive nutrition finding of the 20th century was that periconceptional use (use of the supplement prior to conception and during the first trimester) of a folic-acid containing multivitamin/mineral supplement significantly reduced the occurrence of NTDs. Another key finding was that supplementation with folic acid alone could more than halve the risk of having a second child affected by NTD *(42)*.

The only intervention trial that examined the potential to reduce the risk of first occurrence of NTDs was done in Hungary using a prenatal multivitamin supplement containing 800 µg of folic acid and several other essential micronutrients. The final data set of the Hungarian trial are presented in detail in the Chapter 24 by Czeizel in this volume *(49)* and indicates a significant reduction in two major types of birth defects beyond NTD. Nine cases with defects of the urinary tract were found in the no-multivitamin group and only two were observed in the multivitamin group. The difference was most obvious in the obstructive anomalies of the urinary tract. There were 10 infants with cardiovascular defects in the multivitamin group and 20 in the no-multivitamin group. The difference in the rate of conotruncal heart defects (3 vs 10) was also significant (relative risk [RR] = 0.29, 95% confidence interval [CI] 0.09–0.97). In addition to showing that periconceptional use of multivitamins significantly reduced NTDs, Czeizel and Dudas found that women who took the multivitamin containing 12 vitamins (including 800 µg of folic acid, 4 µg of vitamin B_{12}, 2.6 mg of vitamin B_6, 100 mg of vitamin C, and 15 IU of vitamin E)

also had less morning sickness than the women taking the matched placebo *(50)*. In the UK Medical Research Council (MRC; 1991) Trial for prevention of NTD recurrence, the multivitamin used contained only eight of the vitamins and did not include vitamins E and B_{12}; it only contained 40 mg of vitamin C and 1 mg of vitamin B_6. Although the multivitamin contained 4 mg of folic acid (five times the level in the Czeizel and Dudas trial), there was no decrease in cardiovascular birth defects in either the group taking folic acid alone or the group taking folic acid plus the multivitamins in the MRC Trial. Cardiovascular birth defects were also not lowered in the group taking the multivitamins without folic acid compared to the placebo group *(51)*.

The supplement used in the trial by Czeizel and Dudas is more comparable to the typical one-a-day-type multivitamin/mineral supplement sold in the United States than the supplement used in the MRC Trial. Therefore, it is not surprising that survey data from the United States suggest that lowered cardiovascular birth defect risk is associated with periconceptional use of multivitamin/mineral supplements, which usually contain more of the vitamins and minerals found in the supplement that lowered cardiovascular birth defects.

Because cardiovascular birth defects are about 10-fold more prevalent than NTDs, the total medical costs associated with this defect are high (about $50,000/patient; $3 billion/yr). Fortunately, the risk reduction associated with multivitamin use was high (about 50%) *(49,52)* and, therefore, the potential cost savings approached $1.5 billion per year for hospitalization costs alone *(35)*. The cost savings are particularly great because the balancing costs of the multivitamins are relatively low. Unfortunately, even today, there are few women (about 20%) who take a multivitamin during the periconceptional period before they have confirmed their pregnancy *(53)*.

The cost savings would probably be much greater than $1.5 billion per year because multivitamin use during the months before conception and throughout pregnancy has also been associated with significant reductions in the occurrence of NTDs, renal birth defects, cleft lip and palate, and limb reduction birth defects *(54)*. Supplementation has also been associated with decreased risk of childhood brain tumors *(55)*.

In an editorial in the *New England Journal of Medicine*, Oakley suggested that the right advice to American women is to eat the best diets possible and to take a multivitamin containing folic acid to ensure that the birth-defect preventive level of folic acid is consumed daily *(56)*. This advice was given even after the Food and Drug Administration initiated its policy for fortification of enriched grain products and flour with folic acid. It appears that this level, which raises folic acid intakes by an average of 100 μg per day, may not be sufficient to prevent folic acid-responsive birth defects. Further verification of the need for intake of the 400 μg-level of folic acid is shown in the recent study from China *(57)*. When women anticipating pregnancy took 400 μg of supplemental folic acid during the periconceptional period, there was a fourfold reduction in NTDs in the higher risk area and a 40% reduction in a lower risk area.

2.2. Low Birth Weight and Premature Birth Prevention

Low birth weight and preterm delivery often occur together. Preterm delivery is defined as birth following less than 37 wk of gestation; very preterm delivery is defined as birth following less than 33 wk of gestation. Low birth weight is defined as <2500 g, and very low birth weight is <1500 g. Of all low-birth-weight infants born, 60 to 70% are also preterm. Preterm delivery associated with low birth weight is the second leading cause of infant hospitalization and is also the second leading cause of infant mortality,

following birth defects *(58)*. Low birth weight is second only to cardiovascular birth defects in annual hospitalization costs, exceeding $2.5 billion per year *(35)*. The vast majority of these costs are borne by public funds, and the affected mothers and children are often the least equipped to deal with this event. Preterm births are more prevalent in teenagers, those with less than a high school education, and those with the lowest incomes. In 1995, 7.3% of all infants born in the United States were low birth weight; for African-Americans, the rate was considerably higher (13%; ref. *59*).

In a prospective, case–control study, Scholl et al. *(60)* found that pregnant women in Camden, New Jersey, who took prenatal multivitamins during the first trimester had a fourfold reduction in very preterm births and a twofold reduction in preterm births. Even if supplementation began in the second trimester, there was a significant twofold reduction in very preterm as well as preterm births. The risk of very low birth outcomes (which was highly correlated with preterm delivery) was dramatically reduced by six- to sevenfold when prenatal multivitamins were taken during the first two trimesters. Low birth weight was also reduced significantly with supplementation. These results were observed in women who were at high risk for preterm/low-birth-weight outcomes: they were poor teenagers, and many had low weight gain during pregnancy. Prenatal supplements increased iron and folate status significantly but did not alter serum zinc level. Previously, low iron and/or folate staus has been associated with increased risk of preterm birth and low birth weight *(43)*. Zinc-containing multivitamins were also shown to reduce preterm births in an intervention study *(61)*. Because there was such a dramatic reduction in preterm births in the women who took zinc and (presumably) folic acid-containing prenatal vitamins, the cost savings were predicted to reach over $1.5 billion per year *(35)*. In Chapter 25 of this volume, Scholl *(62)* identifies recent studies where calcium, antioxidants, and long-chain polyunsaturated fatty acids have also reduced the risk of preterm births. It appears that there is significant overlap between the optimal intakes of the nutrients linked to reducing preterm births and those nutrients that reduce the risk of major birth defects. Therefore, such an intervention would be highly cost-effective, saving billions and, perhaps, trillions of dollars every year in global health care costs.

Recently, there have been links made between maternal diet during pregnancy, preterm birth, and CVD in the offspring 50 yr or more after their birth *(45,46)*. We must wait to see if women who used supplements before and during pregnancy had children who, at middle age, have less chronic diseases, such as CVD. Thus, it appears certain that the cost savings associated with a program to provide folic acid-containing multivitamins to all women of childbearing potential both before and during the entire pregnancy would be far greater than those based on either cardiovascular birth defect or preterm birth reduction alone. Moreover, the reduction in pain and suffering for the newborn and parents is substantial.

3. STUDIES OF BIRTH DEFECT AND LOW BIRTH WEIGHT PREVENTION FROM DEVELOPING COUNTRIES

In developing countries, premature births are a serious problem, and an additional problem is intra-uterine growth retardation, which often coincides with premature birth but may also occur with term delivery. DeOnis et al. *(63)* reviewed the 12 nutritionally based interventions that have been examined for their potential to reduce the incidence of intra-uterine growth retardation and, often, preterm birth and low birth weight.

Only one of the interventions (balanced protein/energy supplementation during pregnancy) significantly reduced the risk of low birth weight. The authors also suggested that many of the micronutrient supplementations that were reviewed are likely to prove beneficial, and larger cohorts are required to ensure their efficacy. These micronutrients include vitamin D, folic acid, zinc, calcium, magnesium, and iron. Ramakrishnan *(64)* also extensively reviewed both observational and intervention studies from developing and developed countries that examined the role of micronutrients in optimizing pregnancy outcomes. The authors remind us that in developing countries, one of every five infants (20%) has low birth weight, compared to a rate of 6% in developed countries. Moreover, in developing countries, the majority of low-birth-weight infants are carried to term. Thus, intra-uterine growth retardation is a significant problem in developing countries and impacts the potential physical and, most likely, the mental growth of the child throughout his or her lifetime. Low maternal micronutrient intake of zinc, calcium, magnesium, vitamin A, vitamin C, and, possibly, the B-vitamins copper and selenium is associated with premature birth and low birth weight. Deficiency of iodine, folate, and/or iron is also linked to adverse pregnancy outcomes.

There have been recent data published from two large, well-controlled intervention studies in developing countries. In a placebo-controlled trial, West et al. *(65)* examined the effects of weekly supplementation with the recommended dietary allowance of vitamin A (provided as either preformed vitamin A [retinol] or β-carotene) in over 20,000 pregnant women in Nepal. They found a significant, 40% reduction in maternal mortality with both vitamin A interventions compared to placebo. It is important to note that women were provided the supplements before conception and that this relatively modest dose of vitamin A in the form of β-carotene appeared more effective than the preformed supplement of retinol. Low β-carotene status has been reported in cases of pre-eclampsia in women from developing countries, and as discussed below, the antioxidant potential of β-carotene (compared to the much lower antioxidant potential of retinol) may have been involved in reducing maternal mortality in the study in Tanzania.

The second critical study involved over 1000 pregnant women from Tanzania who were infected with human immunodeficiency virus (HIV) *(66)*. In this placebo-controlled trial, the pregnant women received either placebo; β-carotene and retinol; a multivitamin containing vitamins B_1, B_2, B_6, B_{12}, niacin, folic acid, and vitamins C and E; or the multivitamin plus β-carotene and retinol from 12 to 27 wk of gestation until birth. There was a 40% reduction in fetal death, a 44% reduction in low birth weight, a 39% reduction in very preterm birth, and a 43% reduction in small-for-gestational-age outcomes in the groups supplemented with the multivitamin independent of the vitamin A. Additionally, mothers taking the multivitamin had significantly heavier babies than those not taking the multivitamin ($p = 0.01$). Although the supplement did not contain iron, the women in the multivitamin group had a significant increase in their hemoglobin levels compared to those not taking the multivitamin. Because this study involved HIV-positive women, the investigators also measured the concentration of total T cells (CD3), T-helper cells (CD4), and T-suppressor cells (CD8). Pregnant women who were HIV-positive and took the multivitamin supplement had significant increases in total T cells, which mainly resulted from increases in CD3 cells; CD8 cells also increased. Although vitamin A did not show an effect in this study, it is important to note that the HIV-infected women had low vitamin A status at the onset, and the level provided may not have been sufficient and/or may have not been absorbed sufficiently to show an effect. Vitamin A may also

have been administered too late in these pregnancies to show its effect. In the study by West et al. *(65)*, vitamin A supplementation was started before conception. Vitamin A is critical for early embryonic growth. The critical finding in the study by Fawzi et al. *(66)* was that a multivitamin supplement containing modest doses of micronutrients significantly improved birth outcomes at levels similar to those seen when poor women in the United States who were HIV-negative used multivitamins during their pregnancies *(60)*.

In both developed and underdeveloped countries, there is a direct relationship between the level of poverty and the risk of low-birth-weight outcomes. Ramakrishnan *(67)* rightly argued that improving the nutritional status of poor women prior to and during pregnancy has the potential to significantly reduce the risk of preterm and low-birth-weight births. It is expected that such programs would still be cost-effective, given the many adverse outcomes associated with premature birth and/or low-birth-weight outcomes.

4. REDUCTION IN PRE-ECLAMPSIA AND OTHER PREGNANCY-ASSOCIATED ADVERSE EFFECTS

Pre-eclampsia is defined as hypertension and proteinuria beginning during the second half of gestation. Approximately 5% of pregnant women in the United States develop this condition during pregnancy. Pre-eclampsia is the leading cause of maternal death and accounts for more than 40% of premature births worldwide *(68–70)*. Therefore, the costs associated with pre-eclampsia would include not only 40% of the costs associated with the infant of a preterm delivery but would also include the costs associated with maternal care.

Several mechanisms have been proposed for the initiation and progression of pre-eclampsia, including oxidative damage to the placenta and/or imbalance in blood pressure regulation. Because of the importance of micronutrients such as vitamins C and E (and β-carotene, as mentioned previously) as antioxidants and the association of low calcium status and high blood pressure, these micronutrients have been studied in well-designed protocols to determine their effects on pre-eclampsia.

Bucher et al. *(71)* reviewed 14 randomized clinical trials that involved the use of calcium supplements during pregnancy to determine the effects on maternal blood pressure and pre-eclampsia. They concluded that calcium supplementation (usually 1500–2000 mg/d from week 20 to birth) led to a significant reduction in maternal systolic and diastolic blood pressure and a 62% reduction in the risk of pre-eclampsia. The authors recommended that calcium supplementation be provided for all women at risk for pre-eclamsia. It should be noted that the majority of the populations included in this analysis were not from the United States and had intakes of calcium of about 300 to 500 mg per day—well below their nations' recommended intake levels *(72,73)*.

Several important studies have been published following this review. Levine et al. *(70)* randomized over 4500 pregnant women from the United States to either 2000 mg of calcium or placebo per day and found that there was no difference in the rate of pre-eclampsia between the groups (about 7%). However, it must be noted that these women were not at increased risk for pre-eclampsia and that they had dietary calcium intakes greater than 1100 mg per day—very close to the recommended intake level during pregnancy, yet well above the national average. In a recent re-evaluation of the data concerning calcium and risk of pre-eclampsia, DerSimonian and Levine *(69)* again found that calcium supplementation did not appear to further lower the risk of pre-eclampsia for normal pregnancies but

that for high-risk pregnancies, calcium supplementation appeared to significantly lower the risk. High-risk pregnancies include those of teenagers, women with pre-existing hypertension, and women carrying multiple fetuses. However, the most recent trial studied 1800 mg of elemental calcium supplementation per day in over 400 pregnant women from Australia who had apparently adequate dietary calcium intakes (median: 1100–1200 mg/d) and other characteristics very similar to those in the study by Levine et al. *(70)*. This trial reported a greater than twofold reduction in the risk of pre-eclampsia and a reduction in the risk of preterm delivery from 10% (placebo) to 4.4% (calcium-supplemented). There were also reductions in hospital admissions for threatened preterm labor ($p = 0.03$) and preterm premature rupture of membranes ($p = 0.08$), which would reduce health care costs above and beyond those reduced by the benefits seen with calcium supplementation. In fact, the authors calculated that 73 low-risk women or 42 high-risk women would need to be supplemented to prevent one case of pre-eclampsia. Given that supplementation was given for only 20 wk (at most) and the approximate cost of the calcium supplements are about $20 per woman, it would cost about $1500 to provide the supplements to 73 women—well below the expected medical costs associated with one case of pre-eclampsia and the potential preterm and/or low-birth-weight infant *(74)*.

One other study of interest examined the blood pressure of the children of mothers who took calcium during pregnancy to prevent pre-eclampsia. Belizan et al. *(75)* showed that at age 7 yr, there was a significantly lower systolic blood pressure in the group of children whose mothers had taken 2000 mg of calcium per day during pregnancy. The greatest antihypertensive effect was seen in the overweight children. As mentioned previously, it is not possible to account for all of the savings that might accrue from calcium supplementation if one limits the cost savings to those associated with pre-eclampsia alone. The greatest health care savings may not be seen until decades after the intervention.

As mentioned earlier, oxidative stress is another mechanism that has been examined in pre-eclampsia. Vitamin E is the major lipid-soluble antioxidant in serum, and vitamin C is the major water-soluble antioxidant in the blood. Wang et al. *(76)* found, and Jain and Wise *(77)* confirmed, that serum lipid peroxide levels were significantly higher and serum vitamin E levels were significantly lower in women with pre-eclampsia compared to women with normal pregnancies.

Chappell et al. *(68)* enrolled 283 pregnant women who were at risk for pre-eclampsia in a placebo-controlled trial using daily supplements of vitamin C (1000 mg) and vitamin E (400 IU). There was a significant 61% reduction in risk of pre-eclampsia in the antioxidant-supplemented group. These investigators hypothesized that antioxidants might stabilize the maternal endothelium and placenta and thus reduce risk of pre-eclampsia. They found that the plasma marker for endothelial activation as well as the index for placental dysfunction were both significantly decreased in the supplemented group. The levels of the antioxidants used in this study were well above national recommended intake levels; therefore, this study also provided important data concerning the safety of high doses of antioxidants during pregnancy.

Reduction in pre-eclampsia, which often occurs before term, also reduces the risk of preterm delivery and low-birth-weight outcomes. Again, there appears to be multiple human health benefits as well as billions of dollars of cost-saving benefits that could be realized with the optimization of maternal nutrition based on the recent results from well-controlled clinical trials as well as survey data that associate low intakes of key nutrients with adverse pregnancy outcomes (Table 2).

Table 2
Association of Essential Nutrient Status With Reduced Risk of Adverse Pregnancy Outcomes

Vitamin/mineral	*Low status associated with increased risk*	*Supplementation reduces risk*
Folic acid	Neural tube birth defects Damage to sperm DNA	Neural tube birth defects
Iron	Low birth weight Premature delivery Maternal morbidity and mortality Cognitive deficits	
Calcium	Pre-eclampsia	Pre-eclampsia
Iodine	Cognitive deficits	
Vitamins C and E		Pre-eclampsia
Long chain polyunsaturated fatty acids	Premature delivery	
Vitamin A as retinol and as β-carotene		Low birth weight and premature delivery
Magnesium, copper, selenium, niacin	Premature delivery and low birth weight	
Multivitamin/mineral supplement (60, 62)		Neural tube birth defects, cleft lip/cleft palate, cardiovascular birth defects, renal birth defects, low birth weight, premature delivery
Multivitamin containing zinc (61)		Premature delivery

5. CHILDHOOD

Preventive nutrition strategies in childhood center around two major areas of concern: the effects of severe and marginal deficiencies of essential nutrients in developing countries and the health effects of obesity in the children in developed as well as developing countries. Regarding the former, the UNICEF report in 2004 *(38)* indicated that iron deficiency impairs mental development in young children and is lowering national IQs. It also undermines adult productivity, with estimated losses of 2% of the gross domestic product in the worst affected countries. Vitamin A deficiency compromises the immune systems of approx 40% of children under age 5 yr in the developing world, leading to the deaths of 1 million youngsters each year. Undernutrition of essential vitamins and minerals has its most devastating and durable effects when it occurs early in life and continues through early childhood.

Undernutrition and essential micronutrient deficiencies are not seen exclusively in the underdeveloped countries. For instance, in the United States, the Pediatric Nutrition

Surveillance System, which monitors the nutritional status of low-income children, reported that about 18% of the children under age 2 yr and 17% of children ages 2 to 5 yr had iron deficiency anemia *(59)*. Unfortunately, the percentage of African-American children with anemia actually rose from 21% in 1989 to about 25% in 1997.

In addition to the adverse effects of iron deficiency on cognitive function, deficiencies in iodine, polyunsaturated fatty acids, and vitamin B_{12} have also been linked to decreased cognitive abilities. Other studies have shown that supplementation of well-fed children with zinc, folic acid, vitamins B_6 and B_{12}, iron, or vitamin A improved cognitive function *(78)*.

Childhood obesity is a rapidly growing problem in the United States and worldwide. In Chapter 14 of this volume, Williams *(20)* reviews the demographics and potential treatments currently available. Polhamus et al. *(59)* indicated that the prevalence of excessive weight among children less than age 2 yr is about11%; in children ages 2 to 5 yr, the rate increased from 7% in 1989 to 8.6% in 1997.

In the United States, obesity is found most frequently in populations with the highest poverty rates and lowest levels of education. Drewnowski and Spector *(79)* suggested that high nutrient-dense foods are more expensive and less palatable and that the unexpected rise in obesity levels in the poor resulted partly from poor food choices based on nutritional quality that, in large part, begins early in life and remains the eating pattern into adulthood.

6. THE ADULT CHRONIC DISEASES

There is a critical convergence of the micronutrients that are discussed as preventive nutrition strategies related to pregnancy and those associated with reduction in risk of chronic diseases in adulthood. In fact, the origins of adult chronic disease may be at conception or very soon thereafter, so the similarity in the list of essential nutrients may result from the fact that these are required throughout the life span. It is only now, when there is a critical mass of data, that we are able to see the connecting nutritional threads that run throughout life. Therefore, we now examine the parallel data from studies associating certain nutritional factors with reduction of the risk of adult chronic diseases.

As detailed in Chapter 1 by McGuiness and Ernst, 5 of the 10 leading causes of death in the United States are conditions that can be modified by diet *(80)*. The major broad categories of nutrition-related chronic diseases include CVD, stroke, cancer, diabetes, and osteoporosis. Deterioration of visual and mental/neurological function could also be added to this list *(31)*. In attempting to assign health dollar costs to each of these individual categories, it is evident that these conditions overlap regarding the cost of morbidities and mortalities. For example, a definable comorbidity of obesity is often type 2 diabetes, and the presence of insulin resistance or type 2 diabetes markedly increases risk for CVD *(81)*.

Nevertheless, in the previous edition of Preventive Nutrition, Blumberg reviewed costs that could be attributed to the different chronic diseases *(32)*. Examining the three leading causes of death specifically, he estimated that the annual economic costs of CVD, cancer, and stroke are $138 billion, $104 billion, and $30 billion, respectively, a total exceeding $250 billion annually. Nutritional interventions that could reasonably delay the onset of CVD and stroke for 5 yr could result in annual health cost savings of $84 billion.

6.1. Cardiovascular Disease and Cancer

Bendich et al. *(31)* examined the cost savings that could be accrued if all adults in the United States took a daily supplement containing a minimum of 100 IU of vitamin E.

Based on some of the intervention and epidemiological studies that found significant reductions in risk of heart attack in those who had used vitamin E *(5,82)*, it was predicted that universal intervention could lower annual hospital costs by over $5 billion and lower overall costs associated with CVD (CVD) complications by $20 billion. However, as reviewed in Chapter 10 by Hodis et al. *(82)*, not all recent studies have confirmed secondary cardiovascular risk reduction with supplemental vitamin E. Ongoing studies will help to evaluate the potential for this essential vitamin (with antioxidant and other functions) to affect the primary prevention of CVD.

In addition to antioxidants such as vitamin E, there has also been a continued association of low B-vitamin status (especially folic acid and vitamins B_6 and B_{12}) with increased risk of CVD, stroke, and vascular dementia *(3,34,36,83,84)*. Multivitamin use has been associated with improved B-vitamin status and reduction in homocysteine levels, an important risk factor in CVD and related morbidities *(85)*. Although the cost-effectiveness of multivitamin supplementation has not been reported in the literature, this intervention could also prove to be a cost-saving health strategy.

The potential for supplements to completely halt or slow the progress of already existing disease that has resulted in major debility appears to be quite daunting. Furthermore, the disease may have been progressing for many years before the medically obvious event occurs, and short-term interventions may be just that—too short. Toole et al. *(86)* examined the potential for supplements of vitamins (folic acid and vitamins B_6 and B_{12}) to reduce the risk of recurrent strokes in a 2-yr, double-blind randomized study of 3680 patients who had suffered an ischemic stroke and had higher than average serum homocysteine levels. The comparison was between a multivitamin that included a relatively high level of the three B-vitamins versus one with lower levels. Serum homocysteine levels were moderately lowered in a dose–response manner; the lower the baseline homocysteine, the lower the risk of recurrent stroke. Unfortunately, there was no difference in the rate of recurrent stroke, CHD, or deaths between the groups. The authors suggested that one potential confounder was the mandatory folic acid fortification that occurred during their study. Another possibility is that the study was too short in duration.

McCarron and Heaney *(18)* examined the estimated costs associated with diseases or conditions that can be beneficially affected by a diet that is rich in calcium/dairy products. Direct medical costs associated with obesity and diabetes alone reach $100 billion annually. Osteoporosis costs are about $17 billion, and hypertension adds another $34 billion. Data from intervention studies and recent epidemiological data consistently find a significantly reduced risk of colon cancer associated with higher than average calcium intakes; costs associated with colon cancer alone are estimated at $5 billion annually. Using the findings of the Dietary Approaches to Stop Hypertension intervention study, the epidemiological data from the Coronary Artery Risk Development in Young Adults Study, and several intervention studies using calcium supplements, the authors estimated that $26 billion in health care costs could be saved annually with the inclusion of two additional servings of dairy foods per day; savings would also be seen with increased calcium intake *(18)*.

Another major factor in increasing health care costs is the morbidity associated with obesity. In Chapter 15, Frier and Green suggest that the annual health care costs that can be attributed to obesity from all causes are over $99 billion in the United States *(19)*. Again, the dissection of obesity costs from other factors that are associated with increased CVD and cancer remains to be determined. The associations of obesity with hyperlipidemia, hypertension, and type 2 diabetes all contribute to increased risk for CVD and stroke *(81)*.

Lack of physical activity is certainly another risk factor for both cancer and CVD *(87–89)*. Despite the difficulty in assigning exact dollar costs to each of these risk factors and diseases, it seems obvious that combining nutritional approaches with a healthy lifestyle can have a marked impact on reducing health care costs. However, it has been estimated that only 0.25% of health care cost dollars currently go to prevention strategies that would be extremely cost-effective *(32)*.

6.2. Osteoporosis

Although it is difficult to determine the exact cost savings involved with a preventive nutrition strategy that could impact more than one disease condition, it is worthwhile to examine the "minimal" health economic benefit of implementing such a strategy. We have chosen to examine in detail the data from well-controlled intervention studies that tested the hypothesis that nutritional intervention could reduce the risk of osteoporosis-related hip fracture. We chose this example because there appears to be less overlap between osteoporosis and the other chronic degenerative diseases discussed. However, we are cognizant that the intervention that was common among the studies reviewed, calcium, has been associated with reducing the risk of colon cancer *(90)* and hypertension *(91)*; thus, even in this example, the economic benefits may be underestimated.

More than 6 million adults in the United States have osteoporosis *(92)*, which is defined as having bones that are two standard deviations below the peak bone mineral density (BMD) seen in young adults *(93,94)*. Osteoporosis is a known risk factor for hip fracture *(95)*. Osteoporotic hip fractures pose enormous human and economic costs. The nearly 300,000 annual hip fractures in the United States have been estimated to cost the nation around $5.6 billion based on hospital and other costs. Those with hip fractures experience increased risk of institutionalization and death *(96)*.

Supplemental calcium with or without vitamin D has been shown to reduce the risk of hip fractures *(97–99)*. Three placebo-controlled, double-blind studies showed that calcium supplementation (with or without vitamin D) significantly reduced the risk of hip fracture in individuals over age 50 yr. Bendich et al. *(100)* conducted a meta-analysis from the risk reduction data presented in these three reviews and arrived at a Mantel-Haenzel combined relative risk estimate of 0.53 (95% CI 0.31–0.90) for hip fractures. The pooled relative risk for all nonvertebral fractures, including hip, was 0.61 (95% CI 0.46–0.80). Therefore, in these three studies, there was a 47% reduction in the risk of hip fracture in those individuals who took supplemental calcium at levels that ranged from 500 to 1200 mg per day for up to 3.4 yr. At the same time, there was an additional benefit of a 39% reduction in all types of nonvertebral fractures.

Using the risk reductions seen in these studies and US national data on the hospital and certain other costs associated with hip fracture, the potential reduction in cost was calculated for care of hip fracture patients. Over 134,000 hip fractures and $2.6 billion could have been avoided if all adults over age 50 yr in the United States took 1200 mg of supplemental calcium per day. Universal calcium supplementation was cost-effective for women over age 75 yr based on the need to take the supplemental calcium for 34 mo before the hip fracture reduction was seen. However, if the assumption is made that the benefit would be seen after 12 to 14 mo of supplementation (as was seen in the Chapuy study *[97]*), then universal calcium supplementation becomes cost-effective for all US men and women ages 65 yr and older.

The savings actually are greater because the calculations did not include the cost savings associated with the reduction in the other classes of nonvertebral fractures. The costs

associated with these events are not fully ascertainable, because many of those who break a wrist or other nonvertebral bone often are not hospitalized. Another key finding from this study was that calcium supplementation that commences in women over age 85 yr is very cost-effective, as the intervention data suggest that even at this age, BMD can be lost less quickly if calcium supplementation is instituted.

Although the cost analysis did not include vertebral fractures, it appears that fractures of the spine would also be reduced in many older women who were supplemented with 1200 mg of calcium per day. In a placebo-controlled, double-blind study in women over age 60 yr who consumed less than 1000 mg of calcium per day, Recker et al. *(101)* found that supplementation significantly reduced the risk of spine fractures, especially in women with a history of bone fractures.

The National Institutes of Health and the National Academy of Sciences recommend that postmenopausal women who are not taking estrogen replacement therapy should consume 1500 and 1200 mg, respectively, of elemental calcium daily *(72,73)*. The benefit of estrogen replacement and other antiresorptive therapies for osteoporosis prevention are predicated on the daily consumption of 1000 mg of calcium *(102)*. However, data from a telephone survey of a representative sample of US households revealed that the average daily intake of dietary calcium falls far short of the minimum recommended daily amount of 1000 mg. The telephone survey found that only half of the adults ages 60 to 94 yr drank one glass of milk, which provides 300 mg of calcium, every day *(103)*. LeBoff et al. *(104)* measured the vitamin D and calcium status of postmenopausal women with hip fractures and found that 50% had deficient vitamin D levels, and over 80% had low calcium levels. Because vitamin D is required for calcium absorption, the authors suggested that the low calcium status was linked to the low vitamin D status. Therefore, these data suggest that individuals at risk for hip and other fractures should increase their calcium as well as vitamin D intakes.

Cost savings analyses are based on databases that capture the costs associated with certain events. Thus, when health economists are limited to hospitalization costs, it is not possible to determine the cost savings associated with reducing the occurrence of osteoporosis, because there are no hospital expenses associated with osteoporosis itself. It is well-recognized that increasing bone mass during the years of bone growth and early adulthood is the best preventive strategy for osteoporosis. However, because there are so few hospitalizations of young people who fracture bones, universal calcium (and vitamin D) supplementation may not prove to be cost-effective. Recently, however, Singer et al. *(105)* documented the incidence of fractures in individuals ages 15 to 94 yr in Edinburgh, Scotland. They reported that between the ages of 15 and 49 yr, men had 2.9 times the fractures as age-matched women; fractures of the wrist began to increase in women at age 40 yr, before menopause; and women over age 60 yr had 2.3 times the risk of fractures as men of the same age. Although this study did not examine nutritional factors, other studies have linked increased risk of fractures in young adults with low intakes of calcium and other micronutrients and low sun exposure, as found in Scotland. It may be that the establishment of newer databases that capture the total costs (loss of work as well as medical costs) associated with fractures will permit the evaluation of the cost-effectiveness of earlier nutritional interventions for improvement of bone health.

In addition to reducing the risk of osteoporosis, individuals over age 50 yr who take calcium supplements at the level of about 1200 mg per day may also be lowering their risk for colon cancer. Recently, the data from the Calcium Polyp Prevention Study

(a double-blind, placebo-controlled study) were published *(90)*. The subjects had previously had one colon polyp removed prior to entering this study. Those that took 1200 mg of calcium per day for up to 4 yr had significantly fewer recurrences of polyps than the placebo group. The mechanism of action of calcium in reducing the risk of colon cancer by reducing the number of precancerous lesions is believed to be via the reduction in bile acids that are thought to be carcinogenic.

One other potential benefit of calcium supplementation may be a reduction in blood pressure in both men and women *(91)*. As described earlier, calcium supplementation lowered blood pressure during pregnancy and also reduced blood pressure in the male and female offspring of women who took calcium supplements during pregnancy *(71,75)*. Because high blood pressure is a strong risk factor in stroke and aggravates other CVDs, calcium supplementation may be very cost-effective in all of its biological functions.

It is also important to note that in addition to calcium, antioxidant status has also been associated with risk of hip fracture. Lifestyle factors such as smoking also increase the risk of hip fracture. Melhus et al. *(106)* found a threefold increased risk of hip fracture in women who were current smokers and who had the lowest intakes of either vitamin E or vitamin C compared to nonsmoking women with the highest antioxidant intakes. If the smokers had the lowest intakes of both vitamins, the odds ratio increased to 4.9.

Smoking, independent of antioxidant status, has been shown to increase the risk of hip fracture, which may be a result of its association with decreased calcium absorption *(107)*. To complete the circle-of-life events, it is most interesting to note that Jones et al. *(108)* found that maternal smoking during pregnancy resulted in children with shorter statures that were linked to lower bone mass. Children of smoking mothers may be at greater risk for osteoporosis because their bones never accumulate the bone mass needed to prevent this disease in later life. Importantly, infant bone mass has been shown to be increased if mothers are supplemented with calcium during pregnancy. Koo et al. *(109)* showed that total bone mineral content was significantly greater in infant children born to mothers who were supplemented with 2000 mg of calcium per day during pregnancy compared to women in the placebo group who consumed less than 600 mg of calcium per day.

7. KEY MICRONUTRIENTS FOR DISEASE PREVENTION

Several vitamins and minerals have been consistently associated with primary prevention of the age-related chronic diseases already discussed as well as with reduction in the risk of developing cataracts and age-related macular degeneration reviewed in Chapter 19 by Siegal et al. *(110)*. Coincidentally, the vast majority of these essential micronutrients also appear to the reduce the risk of adverse pregnancy outcomes. The list includes some antioxidant vitamins (vitamins C and E), B-vitamins (folic acid, B_{12}, and B_6), and vitamin D; minerals associated with improved health outcomes for the young and old include calcium, magnesium, iron, zinc, and selenium. It should be noted that iron, zinc, and selenium are also required for the function of antioxidant enzymes. Copper and manganese also are required for some of the antioxidant enzymes, but there are less data currently available concerning the disease-preventing role of these specific minerals.

The majority of data associating micronutrients with primary disease prevention come from epidemiological studies. There appears to be certain chronic diseases that are good candidates for preventive nutrition strategies (Table 3). Findings suggest that individuals with the highest intakes of foods containing these micronutrients have the

Table 3
Diseases/Conditions That Are Good Candidates for Preventive Nutrition Strategies

Disease/conditions—in alphabetical order
Age-related macular degeneration
Birth defects
Cataracts
Colon cancer
Coronary artery disease
Diabetes type II
Hypertension
Kidney stones
Low birth weight
Obesity
Osteoporosis
Pre-eclampsia
Stroke

greatest reduced risk of disease. Some of the survey studies have included questions about dietary supplement use, and, in many cases, those with the highest intakes of the micronutrients from supplements also have lower risks of the major degenerative diseases. Interestingly, often those with the highest intakes of vitamins from naturally occurring sources or from fortified foods also have the highest total intakes because they are often users of vitamin and/or mineral supplements.

It is important to note that for CVD prevention, there appears to be a link between antioxidant nutrients and the B-vitamins *(111)*. Although it is now well-accepted that recommended intake levels (or higher) of folic acid, B_6, and B_{12} are required to lower homocysteine levels, new data suggest that high levels of vitamins E and C may also lower several of the cardiovascular risk factors associated with elevated homocysteine levels. Recent data also indicate that the antioxidant nutrients and the B-vitamins affect different aspects of the cardiovascular system; therefore, both classes of nutrients are needed for protection *(112)*. It appears that in nature, evolution has required man's need for 13 essential vitamins and at least an equal number of minerals. It is only reasonable to find that these dietary components work best when all of their levels are optimized and not just when a "single magic bullet" is at an "optimal" level.

8. CONCLUSIONS AND RECOMMENDATIONS

The examples in this chapter provide ample evidence that there are important, simple, safe, and economically sound interventions that can be implemented to reduce disease risk throughout the life span. Quoting from Blumberg's conclusion *(32)* on "Public Health Implications of Preventive Nutrition," his counsel seems particularly appropriate at this time: "Shifting the health care system from its current emphasis on treatment to prevention will take time. Even if such changes are implemented, more time will be required before its impact on chronic disease mortality will become apparent because of long latency periods, although a delay in the onset of clinical symptoms will be detected earlier. The dividends of prevention in reducing the population illness burden and enhancing of the quality of life can be substantial. Efforts must be strengthened to

encourage all segments of the population to adopt preventive nutrition strategies, not just those who are high risk. Food habits develop early in life, and this is a useful time to develop preventive nutrition behaviors, although an emphasis on older adults appears more critical at this juncture, since by 2004 the cost of health care for those over 65 is projected to constitute 50% of the total national health care bill. Together with an increase in physical activity and the cessation of tobacco use, dietary modification and improvements in nutritional status present us with the greatest potential for reducing the incidence of chronic disease, improving public health, and limiting the growth of health care expenditures."

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Appendix B
Web Sites of Interest

http://www.ifst.org/
The Institute of Food Science & Technology (IFST) is based in the United Kingdom, with members throughout the world, with the purpose of serving the public interest in the application of science and technology for food safety and nutrition as well as furthering the profession of food science and technology. Eligibility for membership can be found at the IFST home page, an index and a search engine are available.

http://www.nysaes.cornell.edu/cifs/start.html
The Cornell Institute of Food Science at Cornell University home page provides information on graduate and undergraduate courses as well as research and extension programs. Links to related sites and newsgroups can be found.

http://www.blonz.com
Created by Ed Blonz, PhD, "The Blonz Guide" focuses on the fields of nutrition, foods, food science and health supplying links and search engines to find quality sources, news, publication, and entertainment sites.

http://www.hnrc.tufts.edu/
The Jean Mayer US Department of Agriculture (USDA) Human Nutrition Research Center on Aging (HNRC) at Tufts University is one of six mission-oriented centers aimed at studying the relationship between human nutrition and health, operated by Tufts University under the USDA. Research programs; seminar and conference information; publications; nutrition, aging, medical and science resources; and related links are available.

http://www.fao.org/
The Food and Agriculture Organization (FAO) is the largest autonomous agency within the United Nations, founded "with a mandate to raise levels of nutrition and standards of living, to improve agricultural productivity, and to better the condition of rural population," emphasizing sustainable agriculture and rural development.

http://www.eatright.org/
The American Dietetic Association is the largest group of food and nutrition professionals in the United States, members are primarily registered dietitians (RDs) and

From: *Preventive Nutrition: The Comprehensive Guide for Health Professionals, Third Edition*
Edited by: A. Bendich and R. J. Deckelbaum © Humana Press Inc., Totowa, NJ

dietetic technicians, registered (DTRs). Programs and services include promoting nutrition information for the public; sponsoring national events, media and marketing programs, and publications (*The American Dietetic Association*); and lobbying for federal legislation. Also available through the website are member services, nutrition resources, news, classifieds, and government affairs. Assistance in finding a dietitian, marketplace news, and links to related sites can also be found.

http://www.faseb.org
The Federation of American Societies for Experimental Biology (FASEB) is a coalition of member societies with the purpose of enhancing the profession of biomedical and life scientists, emphasizing public policy issues. FASEB offers logistical and operational support as well as sponsoring scientific conferences and publications (*The FASEB Journal*).

http://www.foodsciencecentral.com
The International Food Information Service (IFIS) is a leading information, product and service provider for professionals in food science, food technology, and nutrition. IFIS publishing offers a wide range of scientific databases, including FSTA–Food Science and Technology Abstracts. IFIS GmbH offers research, educational training, and seminars.

http://www.ift.org/
The Institute of Food Technologists (IFT) is a membership organization advancing the science and technology of food through the sharing of information; publications include *Food Technology* and *Journal of Food Science*; events include the annual meeting and Food Expo. Members may choose to join a specialized division of expertise (there are 23 divisions); IFT student associations and committees are also available for membership.

http://www.veris-online.org/
The VERIS Research Information Service is a nonprofit corporation, focusing on antioxidants, providing professionals with reliable sources on the role of nutrition in health. Data in VERIS publications, distributed without fee to those who qualify, is based on technical peer-reviewed journals. Quarterly written reports and newsletters, research summaries, annual abstract books, vitamin E fact book and educational programs are among the available VERIS publications and communications. Links to helpful web resources are also accessible.

http://www.osteo.org/
The National Institutes of Health Osteoporosis and Related Bone Diseases–National Resource Center (NIH ORBD-NRC) mission is to "provide patients, health professionals, and the public with an important link to resources and information on metabolic bone diseases, including osteoporosis, Paget's disease of the bone, osteogenesis imperfecta, and hyperparathyroidism. The Center is operated by the National Osteoporosis Foundation, in collaboration with The Paget Foundation and the Osteogenesis Imperfecta Foundation."

http://www.ag.uiuc.edu/~food-lab/nat/
The Nutrition Analysis Tool (NAT) is a free web-based program designed to be used by anyone to analyze the nutrient content of food intake. Links to an "Energy Calculator" and "Soy Food Finder" are also available. NAT is funded by C-FAR at the University of Illinois.

http://www.calciuminfo.com
This is an online information source created, copyrighted, and maintained by GlaxoSmithKline Consumer Healthcare Research and Development. The nutritional and physiological role of calcium is presented in formats designed for health care professionals, consumers, and kids. References and related links, educational games for kids, calcium tutorials, and a calcium calculator are easily accessible.

http://vm.cfsan.fda.gov/
The Center for Food Safety and Applied Nutrition (CFSAN) is one of five product-oriented centers implementing the Food and Drug Administration's mission to regulate domestic and imported food as well as cosmetics. An overview of CFSAN activities can be found along with useful sources for researching various topics such as food biotechnology and seafood safety. Special interest areas, for example, advice for consumers, women's health, and links to other agencies are also available.

http://www.bcm.tmc.edu/cnrc/
The Children's Nutrition Research Center (CNRC) at Baylor College of Medicine is one of six USDA/ARS human nutrition research centers in the nation, assisting health care professionals and policy advisors to make appropriate dietary recommendations. CNRC focuses on the nutrition needs of children, from conception through adolescence, and of pregnant and nursing women. Consumer news, seminars, events, and media information are some of the sections available from this home page.

http://www.dsqi.org/
The Dietary Supplement Quality Initiative (DSQI) is designed to educate consumers on the health benefits, safety, standards and regulations, and labeling of dietary supplements. Industry news, interviews, editorials, and DSQI resources and services provide useful tools for consumers, practitioners, producers and distributors.

http://www.usda.gov
The US Department of Agriculture (USDA) provides a broad scope of service to the nation's farmers and ranchers. In addition, the USDA ensures open markets for agricultural products, food safety, environmental protection, conservation of forests and rural land, and the research of human nutrition. Affiliated agencies, services and programs are accessible through this web site.

http://www.nalusda.gov/
The National Agriculture Library (NAL), a primary resource for agriculture information, is one of four national libraries in the United States and a component of the Agriculture Research Service of the US Department of Agriculture. Access to NAL's institutions and resources are available through this site.

http://www.fns.usda.gov/fns/
The Food and Nutrition Service (FNS) administers the US Department of Agriculture's (USDA) 15 food assistance programs for children and needy families with the mission to reduce hunger and food insecurity. Details of nutrition assistance programs and related links can be found.

http://www.agnic.org/
The Agriculture Network Information Center (AgNIC), established through the alliance of the National Agriculture Library (NAL) and other organizations, provides public access to agriculture-related resources.

http://www.who.int/nut/welcome.htm
The World Health Organization (WHO) has regarded nutrition to be of fundamental importance for overall health and sustainable development. The global priority of nutritional issues, activities, mandates, resources, and research are presented in detail.

Nutritional Science Journals

Brown CM. Where to find nutritional science journals on the World Wide Web. J Nutr 1997; 127:1527–1532.

http://www.crcpress.com/jour/catalog/foods.htm
Critical Reviews in Food Science and Nutrition

http://www.wiley.com/Home.html
International Journal of Eating Disorders

http://www.peakcom.com/clinnutr.org/jabs.html
Journal of Parenteral and Enteral Nutrition

http://www.lrpub.com/journals/j1013.htm
Journal of Pediatric Gastroenterology and Nutrition

http://www.elsevier.nl:80/inca/publications/store/5/2/5/0/1/3/
Journal of Nutritional Biochemistry

http://www.karger.com/journals/anm/anm jh.htm
Annals of Nutrition and Metabolism

http://www.hscsyr.edu/ñutrition/
Nutrition: The International Journal of Applied and Basic Nutritional Sciences

http://www.elsevier.nl/inca/publications/store/5/2/5/4/8/3/
Nutrition Research

http://www.humanapress.com
Humana Press web site

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